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Antioxidant Content in Low Density Lipoprotein and Lipoprotein Oxidation *In Vivo* and *In Vitro*

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Human blood contains naturally occurring multiple-modified low density lipoprotein (nomLDL) capable of inducing the accumulation of cholesteryl esters in the cells of human aortic intima. NomLDL is desialylated particles of small size with an increased electronegative charge which can be separated from native low density lipoprotein (LDL) by lectin chromatography. The purpose of this study was to determine the content of antioxidants in native and nomLDL obtained from healthy subjects and from patients with coronary heart disease as well as to elucidate a possible relationship between the level of antioxidants and the degree of *in vivo* and *in vitro* LDL oxidizability. The apoB-bound cholesterol level in native and nomLDL of healthy subjects was 0.25 ± 0.08 and 0.28 ± 0.05 mol/mol apoB, respectively. The level of apoB-bound cholesterol in native LDL of coronary atherosclerosis patients showed no significant difference from that in healthy subjects' native lipoprotein. At the same time, the level of apoB-bound cholesterol in patients' nomLDL was 7-fold higher than in native LDL. The average duration of the lag phase of native LDL oxidation did not show a significant difference between the lipoprotein of healthy subjects and coronary atherosclerosis patients. The lag phase of nomLDL obtained from healthy subjects and patients was significantly shorter (3- and 6-fold, respectively) than for their native LDL. The latter finding points to their increased susceptibility to *in vitro* oxidation. Oxidizability of total LDL preparations correlated positively with their nomLDL content. The content of all the antioxidants studied (coenzyme-Q_{10}, α- and γ-tocopherols, β-carotene and lycopene) in nomLDL was 1.5- to 2-fold lower than in native LDL. The level of apoB-bound cholesterol in nomLDL, correlated positively with the ubiquinone-10 content and showed negative correlation with ubiquinol-10 and β-carotene levels. On the other hand, the content of apoB-bound cholesterol in native LDL correlated positively with the ubiquinol-10 level. Susceptibility of nomLDL to *in vitro* oxidation exhibited negative correlation with α-tocopherol and β-carotene levels and a positive correlation with the ubiquinone-10 content. On the contrary, oxidizability of native LDL correlated positively with the ubiquinone-10 level. Conclusions: (a) elevated apoB-bound cholesterol level in nomLDL of coronary atherosclerosis patients indicates that peroxidation of lipids occurs *in vivo*; (b) *in vivo* lipoperoxidation in nomLDL is corroborated by increased proportion of oxidized form of coenzyme-Q_{10}; (c) content of lipid-soluble antioxidants in nomLDL is lower than in native lipoprotein; (d) nomLDL has a higher susceptibility to *in vitro* oxidation than native LDL; (e) it is necessary to use isolated subfractions of native LDL and nomLDL, but not total lipoprotein preparations, to study the mechanisms of lipid peroxidation.

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INTRODUCTION

According to the currently widespread view, oxidative modification of low density lipoprotein (LDL) plays a key role in atherogenesis.[1] However, oxidized LDL has not yet been found in the bloodstream. Several types of modified LDL were isolated from human blood plasma, namely, electronegative, small/dense and multiple-modified (desialylated) LDL.[2–4] Naturally occurring multiple-modified LDL (nomLDL) is characterized by a low content of sialic acid and neutral saccharides, decreased level of neutral lipids and phospholipids, altered tertiary structure of apolipoprotein B (apoB) and aggregation of lipoprotein particles as well as the ability to interact with the scavenger receptor and cellular asialoglycoprotein receptor. All these properties along with a low rate of the intracellular LDL degradation account for its ability to induce the accumulation of lipids in smooth muscle cells of human aortic intima and macrophages.[5–8] As far as their physical parameters are concerned, nomLDL is small dense particles possessing an increased electronegative charge[6] and is characterized by an increased susceptibility to *in vitro* oxidation.[6]

We have recently shown that lipid peroxidation processes occurring in LDL lead to the formation of lipid adducts with apoB.[9] The lipids covalently bound to apolipoprotein cannot leave the lipoprotein particle in circulation and are retained during isolation of LDL representing a reliable marker of lipoperoxidation. The level of apoB-bound cholesterol in multiple-modified desialylated LDL is higher than in native LDL.[9]

In this study, the LDL content of apoB-bound cholesterol, i.e. the degree of their *in vivo* oxidation, and LDL *in vitro* oxidation is related to the lipoprotein antioxidant levels.

MATERIALS AND METHODS

Donors

Blood from healthy subjects (30 males aged 30 to 45 years) and from patients with coronary atherosclerosis (30 males aged 30 to 54 years) was analysed. Healthy subjects had no family history of premature coronary artery disease and had no complaints and signs of myocardial ischemia on a bicycle exercise test. Patients had effort angina pectoris of the II–IV functional class according to Campeau.[10] The degree of stenosis of 1–3 coronary arteries assessed by selective coronarography was 75% or higher. None of the donors had diabetes mellitus or arterial hypertension. Fourteen of 30 healthy subjects and 12 of 30 patients were smokers. In both groups, the cholesterol level of the blood plasma was < 200 mg/dl, triglyceride level < 150 mg/dl. None of the 60 donors took antioxidant supplementation or hypolipidemic drug therapy. LDL samples of ten healthy subjects and ten patients were randomly selected to evaluate the degree of *in vivo* and *in vitro* oxidizability.

LDL Isolation

Patients were fasted overnight (12 h) and blood was drawn in the morning into plastic tubes containing 1 mg/ml EDTA. Blood plasma was obtained by centrifugation for 10 min at 3000 rpm. LDL was isolated by a two-step ultracentrifugation as described earlier.[9] The amount of nomLDL in total LDL fraction was evaluated by lectin sorbent assay as described elsewhere.[11] For separation of native and desialylated nomLDL, 10–15 mg total LDL was applied on 5 ml RCA_{120}-agarose column.[12] The column was washed sequentially with 50 ml PBS and 25 ml PBS containing 0.5 M NaCl. Bound LDL fraction was eluted from the RCA_{120}-agarose column with 50 mM galactose in PBS. Unbound (native LDL) and bound (desialylated nomLDL) fractions were collected, adjusted to proper density with solid NaBr and concentrated by

ultracentrifugation. LDL was dialyzed at 4°C overnight in darkness against 2000 volumes of PBS containing 0.01 mM EDTA. EDTA was omitted from PBS when LDL samples were subjected to *in vitro* oxidation. Protein content was measured according to Lowry *et al.*[13] using bovine serum albumin as a standard. NomLDL levels in total LDL preparations of healthy subjects and patients were $10 \pm 2\%$ and $45 \pm 5\%$, respectively. LDL samples (150 µg protein/ml) were oxidized at 37°C in presence 10 µM $CuSO_4$. The content of conjugated dienes was determined as described by Esterbauer *et al.*[14]

Antioxidant Determination

Lipoprotein lipids were extracted by methanol/hexane system as described by Yamamoto *et al.*[15] Lipoprotein solution (200–400 µl) was extracted with 2 ml of methanol, containing 0.02% acetic acid and 10 ml hexane and dried hexane was redissolved in 50 µl of the solvent system used subsequently as mobile phase for the high performance liquid chromatography (HPLC) separation. To determine content of tocopherols, ubiquinone-10 and ubiquinol-10, a volume of 20 µl was separated on a Supelcosil LC-18 column (250 × 4.6 mm, 5 µm particle size, Supelco Inc., Bellefonte, PA) with ethanol/methanol (3:1, v/v). Content of γ-tocopherol, α-tocopherol and ubiquinol-10, that eluted at 5.6, 7.2, and 21.3 min, respectively, were determined by UV detection at 295 nm. Ubiquinone-10 (elution time – 31.0 min) was detected at 275 nm. To obtain ubiquinol-10, standard ubiquinone-10 was treated with sodium borohydride as described by Frei *et al.*[16] Carotenoid level was determined by HPLC chromatography with detection at 450 nm according to Ziouzenkova *et al.*[17]

The intraassay CVs and interassay CVs were estimated by repetition of the measuring of the same plasma sample and involved 6 injection for each kind of analysis (tocopherols/coenzyme-Q_{10} and carotenoids) in one day and 12 injections in 4 different days, respectively. Intra- and interassay CVs were 3.7% and 5.9%, 5.6% and 7.8%, 4.5% and 9.7% for α-tocopherol, ubiquinone-10 and β-carotene, respectively. Stability of antioxidants was evaluated by 6 measurements of the same LDL extract sample in 6 h period of time. CVs for all investigated compounds were lower than 5%. The recovery of antioxidants, defined as the increase measured when known amounts of compound were added to serum, ranged from 95% to 104%.

Determination of apoB-bound Cholesterol

To obtain delipidated apolipoprotein B (apoB), LDL samples (1 ml, 0.5–1.5 mg/ml) were extracted two times with 2 ml of isopropanol, five times with 4 ml of chloroform–methanol mixture (1:2, v/v) and twice with 2 ml of diethyl ether.[9] Delipidated apoB was dissolved in 0.2–0.5 ml 0.1 M NaOH. Protein content was measured according to Lowry *et al.*[13] To measure apoB-bound cholesterol, 100 µl of sample were heated for 30 min at 100°C, cooled and extracted twice with 1 ml chloroform. Extracts were evaporated and lipids were dissolved in methanol. Cholesterol content was determined by high-performance liquid chromatography on the Supelcosil LC-18 column (250 × 4.6 mm, 5 µm particle size) using methanol : water (95:5, v/v) as solvent, UV detection at 210 nm.

Statistical Analysis

The significance of differences between group mean values was evaluated by multiple t-test of one-way analysis of variance using a BMDP statistical program package.[18] Significance of the correlation coefficient was evaluated using Fisher's Z-transformation.[19]

RESULTS

The Level of apoB-associated Cholesterol in Native LDL and nomLDL

Table I shows the data on the level of apoB-bound cholesterol in native LDL and nomLDL isolated

from the blood plasma of healthy subjects and patients with coronary atherosclerosis. The content of apoB-associated cholesterol in native LDL of healthy subjects was 0.25 mol/mol, i.e. one cholesterol molecule per 4 lipoprotein particles, on average. The apoB-bound cholesterol levels of native LDL and nomLDL of healthy subjects were similar (Table I).

The content of apoB-bound cholesterol in native LDL of coronary atherosclerosis patients showed no significant difference from that of healthy subjects' native LDL (Table I). At the same time, nomLDL of patients contained 7-fold more apoB-bound cholesterol as compared with native LDL. Thus, nomLDL of patients with coronary atherosclerosis reveals a higher degree of oxidation *in vivo* than native LDL.

TABLE I Level of apoB-bound cholesterol and susceptibility to Cu^{2+}-mediated oxidation (lag phase) of native and nomLDL of healthy subjects and patients

LDL subfraction	Level of apoB-bound cholesterol (mol/mol apoB)	Lag phase (min)
Healthy subjects ($n = 10$)		
Native LDL	0.25 ± 0.08	103 ± 16
NomLDL	0.28 ± 0.05	34 ± 3*
Coronary atherosclerosis patients ($n = 10$)		
Native LDL	0.16 ± 0.05	142 ± 13
NomLDL	1.18 ± 0.33*,†	25 ± 2*,†

Data represent means of 10 determinations ± SEM.
*Significant difference from native LDL, $p < 0.05$. †Significant difference from healthy subjects, $p < 0.05$.

Susceptibility of Native LDL and nomLDL to *In Vitro* Oxidation

Lipoprotein susceptibility to *in vitro* oxidation was evaluated by the duration of lag phase during Cu^{2+}-stimulated oxidation (Table I). It was demonstrated that an average duration of the lag phase during oxidation of healthy subjects' nomLDL is 3-fold shorter as compared with native lipoprotein, i.e. oxidizability of modified lipoproteins is considerably higher. The lag phase of coronary atherosclerosis patients' nomLDL, on average, was 5.7-fold shorter than that of native LDL (Table I). Moreover, the average duration of the lag phase during oxidation of patients' nomLDL was 1.4-fold smaller than that of healthy subjects' nomLDL. Thus, nom LDL of both healthy subjects and, especially, coronary atherosclerosis patients exhibit an increased susceptibility to *in vitro* oxidation as compared with native LDL. In addition to the LDL subfractions, this study revealed susceptibility to *in vitro* oxidation in total LDL preparations of healthy subjects and patients. Average duration of the lag phase during oxidation of total LDL of healthy subjects and coronary atherosclerosis patients was 82 ± 6 and 58 ± 12 min ($n = 20$, $p < 0.05$), respectively.

The Content of Antioxidants in Native LDL and nomLDL

Table II shows the data on the content of oxidized (ubiquinone-10, CoQ_{10}) and reduced

TABLE II Content of ubiquinone-10 (CoQ_{10}) and ubiquinol-10 ($CoQ_{10}H_2$) in native and nomLDL of healthy subjects and coronary atherosclerosis patients

LDL subfraction	CoQ_{10} content (mmol/mol apoB)	$CoQ_{10}H_2$ content (mmol/mol apoB)	$CoQ_{10}/CoQ_{10} + CoQ_{10}H_2$
Healthy subjects ($n = 30$)			
Native LDL	76 ± 5	718 ± 45	0.10
NomLDL	92 ± 5*	512 ± 41*	0.15
Coronary atherosclerosis patients ($n = 30$)			
Native LDL	122 ± 12†	336 ± 31†	0.27
NomLDL	124 ± 11†	222 ± 30*,†	0.36

Data represent means of 30 determinations ± SEM. *Significant difference from native LDL, $p < 0.05$. †Significant difference from healthy subjects, $p < 0.05$.

(ubiquinol-10, $CoQ_{10}H_2$) forms of coenzyme-Q_{10} in native LDL and nomLDL of healthy subjects and coronary atherosclerosis patients. The ubiquinone-10 content in nomLDL of healthy subjects, on average, was 21% higher than in native LDL while their ubiquinol-10 content was 29% lower than that of native lipoproteins (Table II). The proportion of oxidized form of coenzyme-Q_{10} in nomLDL 1.5-fold exceeded that in native LDL isolated from the blood of healthy subjects.

The level of ubiquinone-10 in native LDL of coronary atherosclerosis patients, was 1.6-fold higher than in native lipoproteins of healthy subjects (Table II). The ubiquinone-10 content in patients' nomLDL, was 35% higher than in nomLDL of healthy subjects. The ubiquinol-10 level in native LDL of coronary atherosclerosis patients was 2.1-fold lower than in healthy subjects' native LDL. Even a lower content of ubiquinol-10 was found in nomLDL of coronary atherosclerosis patients (Table II). The percentage of an oxidized form of coenzyme-Q_{10} in native LDL and nomLDL of patients was 2.7- and 2.4-fold higher than in the respective lipoprotein subfractions of healthy subjects.

Table III shows the data on the content of α- and γ-tocopherols in native LDL and nomLDL of healthy subjects and coronary atherosclerosis patients. The level of both α- and γ-tocopherols in healthy subjects' nomLDL was respectively 1.5- and 1.6-fold lower than in native LDL.

TABLE III Content of α-tocopherol and γ-tocopherol in native LDL and nomLDL of healthy subjects and coronary atherosclerosis patients

LDL subfraction	α-Tocopherol content (mol/mol apoB)	γ-Tocopherol content (mol/mol apoB)
Healthy subjects ($n=30$)		
Native LDL	8.21 ± 0.35	0.49 ± 0.02
NomLDL	5.43 ± 0.31*	0.31 ± 0.02*
Coronary atherosclerosis patients ($n=30$)		
Native LDL	7.99 ± 0.36	0.43 ± 0.02†
NomLDL	5.66 ± 0.41*	0.29 ± 0.01*

Data represent means of 30 determinations ± SEM.
*Significant difference from native LDL, $p < 0.05$. †Significant difference from healthy subjects, $p < 0.05$.

The content of α-tocopherol in native LDL of coronary atherosclerosis patients was similar to that of the healthy subjects' native LDL (Table III). The content of γ-tocopherol in native LDL of patients was significantly lower (by 12%) than in native LDL of healthy subjects. The α- and γ-tocopherol levels in patients' nomLDL were respectively 1.4- and 1.5-fold lower than in native LDL.

The levels of β-carotene and lycopene in the LDL subfractions of healthy subjects and coronary atherosclerosis patients are presented in Table IV. The content of β-carotene in native LDL of healthy subjects and patients with coronary atherosclerosis showed no significant differences while in nomLDL of both healthy subjects and patients it was 1.3-fold lower than in native LDL (Table IV).

The lycopene content in native LDL of healthy subjects and coronary atherosclerosis patients showed no significant differences and amounted to 201 and 186 mmol/mol apoB, respectively (Table IV). The lycopene levels in nomLDL of healthy subjects and patients were respectively 1.4- and 1.3-fold lower as compared with native lipoprotein.

Relationship between Antioxidant Levels and Degree of LDL *In Vivo* Oxidation and LDL Susceptibility to *In Vitro* Oxidation

Table V shows the coefficients of correlation between the LDL antioxidant levels and the

TABLE IV Content of β-carotene and lycopene in native LDL and nomLDL of healthy subjects and coronary atherosclerosis patients

LDL subfraction	β-Carotene content (mmol/mol apoB)	Lycopene content (mmol/mol apoB)
Healthy subjects ($n=30$)		
Native LDL	345 ± 16	201 ± 15
NomLDL	263 ± 13*	145 ± 9*
Coronary atherosclerosis patients ($n=30$)		
Native LDL	318 ± 23	186 ± 16
NomLDL	238 ± 16*	140 ± 11*

Data represent means of 30 determinations ± SEM.
*Significant difference from native LDL, $p < 0.05$.

TABLE V Correlation between apoB-bound cholesterol or lag phase and antioxidants in LDL

Antioxidant	apoB-bound cholesterol		Lag phase	
	Native LDL	NomLDL	Native LDL	NomLDL
α-Tocopherol	0.32	−0.23	0.20	0.55*
γ-Tocopherol	0.20	−0.04	0.26	0.31
CoQ_{10}	−0.14	0.55*	0.63*	−0.46*
$CoQ_{10}H_2$	0.50*	−0.40*	−0.32	0.18
$CoQ_{10} + CoQ_{10}H_2$	0.49*	−0.31	−0.23	0.11
$CoQ_{10}/CoQ_{10} + CoQ_{10}H_2$	−0.32	0.63*	0.41*	−0.50*
β-Carotene	0.28	−0.51*	−0.09	0.60*
Lycopene	−0.03	−0.19	−0.06	0.07
Lag phase	−0.26	−0.61*	1.00	1.00

* $p < 0.05$.

content of apoB-bound cholesterol which determines the degree of lipoprotein oxidation *in vivo*. Besides, it gives a correlation between the antioxidant content and duration of the lag phase reflecting the susceptibility of LDL to *in vitro* oxidation. The content of apoB-bound cholesterol in native LDL of healthy subjects and coronary atherosclerosis patients correlated significantly with the content of ubiquinol-10 as well as total coenzyme-Q level. On the other hand, the content of apoB-bound cholesterol in nomLDL correlated positively with the absolute content and percentage of ubiquinone-10 and showed negative correlation with the ubiquinol-10 level (Table V). Negative correlation was also found between the level of apoB-bound cholesterol and the content of β-carotene in nomLDL.

The level of apoB-bound cholesterol in nomLDL correlated negatively with the duration of the lag phase during *in vitro* oxidation of LDL.

The duration of the lag phase during a copper-dependent oxidation of native LDL correlated positively with the quantity and percentage of oxidized coenzyme-Q_{10} (Table V). On the contrary, the duration of the lag phase of nomLDL oxidation exhibited a significant negative correlation with these two parameters. Significant correlation was also revealed between the duration of the lag phase at nomLDL oxidation and the levels of α-tocopherol and β-carotene (Table V).

The levels of γ-tocopherol and lycopene in native LDL and nomLDL did not correlate either with the apoB-bound cholesterol level or duration of the lag phase of lipoprotein oxidation (Table V).

The duration of the lag phase of the copper-dependent oxidation of total LDL preparations obtained from the blood of healthy subjects and CHD patients correlated negatively with their nomLDL percentage ($r = -0.68$, $n = 20$, $p < 0.05$).

DISCUSSION

The data obtained in this study indicate that the level of apoB-bound cholesterol in nomLDL of patients with coronary atherosclerosis is, on average, several times higher than in native LDL. As was pointed out previously, lipid adducts with the apoprotein formed due to lipoperoxidation cannot leave the lipoprotein particle and, hence, their level reflects the intensity of lipid peroxidation processes which have occurred in the LDL *in vivo*. Thus, the results of this study coincide with the previously obtained data[9] and, apparently, represent the first convincing evidence that lipoperoxidation takes place in one of the subfractions of LDL circulating in human blood. As follows from our data, nomLDL are more oxidized than native LDL.

An increased quota of ubiquinone-10 in nomLDL as compared with native LDL is another proof of lipoperoxidation occurring in these particles. It should be pointed out that the total ubiquinone-10 and ubiquinol-10 content of healthy subjects' native LDL approximates to one molecule per lipoprotein particle, and only 10% of the antioxidant is oxidized. The total ubiquinone-10 and ubiquinol-10 content in nomLDL of both healthy subjects and coronary atherosclerosis patients is significantly lower than in native LDL which, apparently, reflects the loss of lipids by modified LDL particles.[6] However, the proportion of the oxidized form of ubiquinone-10 in nomLDL of both healthy subjects (15%) and patients (36%) is higher than in native LDL.

Both findings indicating that lipid peroxidation may take place in nomLDL *in vivo* in all probability represent two interrelated events. Indeed, our data show that the content of apoB-associated cholesterol in nomLDL correlates positively with the absolute and relative ubiquinone-10 content and shows negative correlation with the content of the reduced form of the antioxidant. It can be assumed that lipid peroxidation processes in modified LDL are accompanied both with a fall of the reductive coenzyme-Q_{10} form level and accumulation of the oxidized form of coenzyme-Q_{10}, and a rise in the apoB-bound cholesterol content.

In native LDL the levels of apoB-bound cholesterol, ubiquinone-10 and ubiquinol-10 exhibited a contrary relationship. Thus, we were surprised to find positive and close correlation between the content of apoB-bound cholesterol in native LDL and their level of ubiquinol-10 and total coenzyme-Q_{10} level. Moreover, it was found the presence of positive correlation between the duration of the lag phase of the copper-dependent oxidation of native LDL and their absolute and relative ubiquinone-10 content. One can assume that coenzyme-Q_{10} performs a prooxidant function in isolated native LDL in certain conditions (high levels of ubiquinol-10 and polyunsaturated fatty acids, a substrate for peroxidation, high concentrations of transition metals etc.). However, further studies on preparations of native LDL purified of modified LDL are required.

The duration of the lag phase of copper-dependent oxidation of nomLDL correlated positively with their α-tocopherol and β-carotene content which indicates that these antioxidants play a protective role in lipid peroxidation *in vitro*. An important role of α-tocopherol in the inhibition of peroxidation of LDL lipids is well documented.[20–22] Esterbauer *et al.*[23] showed that duration of the lag phase of copper-dependent oxidation of CHD patients' total LDL, which were not subjected to vitamin E load, did not correlate with the content of α-tocopherol in the lipoprotein. An absence of correlation between the level of α-tocopherol in LDL and its susceptibility to oxidation induced by γ-irradiation or incubation with macrophages was also demonstrated by other authors,[24,25] as was the absence of correlation between the level of β-carotene and oxidizability of patients' total LDL free from β-carotene load.[26,28] In our experiments, we also failed to find interrelationship between the levels of α-tocopherol and β-carotene, and oxidizability of total LDL isolated from the blood plasma of healthy subjects and coronary atherosclerosis patients (Tertov, V., Orekhov, A., unpublished work). The negative correlation between the content of exogenous antioxidants and oxidizability of nomLDL allows to assume that these agents play a protective role regarding lipid peroxidation after oxidation of ubiquinol-10 to ubiquinone-10.

The mechanism of the antioxidant action of β-carotene in LDL is not fully clear. β-Carotene is able to quench singlet oxygen[28] and acts as a chain-breaking antioxidant.[29] According to Wagner *et al.*, in LDL β-carotene is one of the most effective quenchers of singlet oxygen.[30] The fact that the content of apoB-bound cholesterol in nomLDL correlated negatively with their

level of β-carotene suggests an important role of singlet oxygen in the formation of lipid adducts with apoB. The content of other carotenoids (α-carotene, lutein, zeaxanthin, canthaxanthin, cryptoxanthin) was below 100 mmol/mol apoB (i.e. less than one molecule per 10 particles) and did not correlate either with the degree of oxidation or LDL oxidizability and, hence, is not adduced in this report.

In this study, we observed a negative correlation between the duration of the lag phase of copper-dependent oxidation of nomLDL and their content of apoB-bound cholesterol, indicating that peroxidation of lipids *in vivo* makes lipoproteins more susceptible to oxidation *in vitro*.

Furthermore, the susceptibility of total LDL to *in vitro* oxidation correlates positively with the proportion of nomLDL in the isolated preparations. Variable resistance to *in vitro* oxidation exhibited by healthy subjects' LDL of different density was demonstrated by Tribble *et al.*[31] It was also shown that the fraction of dense LDL is characterized by decreased α-tocopherol and ubiquinol-10 levels. Increased susceptibility of dense LDL to *in vitro* oxidation was also revealed by other researchers.[32–34] Avogaro *et al.*[35] demonstrated that the subfraction of electronegative LDL isolated by ion-exchange chromatography has a two-fold lower tocopherol content as compared with native lipoproteins. It was also shown that electronegative LDL has a higher susceptibility to *in vitro* oxidation.[36] Moreover, susceptibility of total LDL to *in vitro* oxidation directly correlated with their percentage of electronegative LDL. Increased oxidizability of the electronegative LDL subfraction was also reported by Shimano *et al.*[37] We have recently shown that naturally occurring multiple-modified (desialylated) LDL, small dense LDL and electronegative LDL have similar physical properties and chemical composition, and represent the same lipoprotein particles which underwent multiple modification.[38,39] The results of this study provide an additional evidence confirming this hypothesis. At the same time, obtained data show that investigation of mechanisms lipid peroxidation and antioxidant action should be done using isolated LDL subfractions.

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