Radical-Mediated Oxidation of Isolated Human Very-Low-Density Lipoprotein

Dette Mohr, Roland Stocker

Abstract Oxidative modification of human low-density lipoprotein (LDL) has received much attention because of its suggested involvement in the early events of atherogenesis. In contrast, little data exist concerning the oxidation of human very-low-density lipoprotein (VLDL), although such modification promotes foam cell formation by these lipoproteins. We therefore investigated the radical-mediated oxidation of VLDL by using controlled oxidizing conditions and sensitive and specific methods to assess lipoprotein lipid oxidation and antioxidation. We observed that the ratio of α-tocopherol to coenzyme Q9 in VLDL was close to that of LDL, suggesting that these lipoproteins may transport some coenzyme Q9 to extrahepatic tissues, as they do tocopherol. Most of the coenzyme Q9 associated with VLDL was present in its reduced, antioxidant active form, ubiquinol-10. The small amounts of ubiquinol-10 in VLDL provided the lipoprotein lipids with a highly efficient antioxidant protection. Also, the kinetics of radical-mediated lipid peroxidation in VLDL resembled that in LDL and therefore also probably proceeded via the recently described tocopherol-mediated peroxidation mechanism. Oxidation competition experiments using aqueous radicals and physiological concentrations and molar ratios of LDL and VLDL indicated that in contrast to the situation with high-density lipoproteins, lipid peroxidation was initiated and detected simultaneously in the former two lipoprotein particles. However, once initiated, peroxidation propagated at an approximately twofold higher rate in VLDL than LDL. Our studies suggest that radical-mediated lipid (per)oxidation proceeds via similar mechanisms in isolated LDL and VLDL. We conclude that efficient LDL antioxidants are also likely to be effective protective agents for VLDL. (Arterioscler Thromb. 1994;14:1186-1192.)

Key Words • atherosclerosis • free radicals • triglycerides • lipid peroxidation • ubiquinol • antioxidants

Low-density lipoproteins (LDLs) are generally considered to promote atherosclerosis, whereas high-density lipoproteins (HDLs), which are involved in reverse cholesterol transport, are considered to be antiatherogenic. The proatherogenic action of LDL is associated with its modification, which can result in rapid and uncontrolled uptake of the lipoprotein by certain cells (eg, macrophages and smooth muscle cells) with concomitant transformation of the latter into lipid-laden or foam cells. While the precise molecular mechanisms underlying its modification remain to be elucidated, oxidation of LDL is now generally believed to be important for the in vivo formation of foam cells. Other modifications, such as those resulting from exposure to and interaction of LDL with human arterial proteoglycans, may also contribute to the in vivo foam cell formation either separately or in combination with oxidative modification.

The atherogenic risk associated with high levels of very-low-density lipoproteins (VLDLs) remains controversial. However, oxidatively modified VLDL, similar to LDL, may contribute to in vivo foam cell formation in humans. This notion is based on the observation that in vitro exposure of β-VLDL isolated from animals fed a cholesterol-rich diet to macrophages or smooth muscle cells caused oxidation of this lipoprotein, resulting in its rapid uptake and a subsequent massive intracellular accumulation of lipids. Uptake of such oxidatively modified VLDL by macrophages is not mediated by the LDL receptor but rather via a receptor similar if not identical to that for oxidized LDL. Because of its potential relevance to atherogenesis, it is important to understand the mechanism(s) of VLDL oxidation and how this process is inhibited by the antioxidants associated with this lipoprotein.

Studies on oxidative modification of VLDL have been carried out predominantly, if not exclusively, with β-VLDL, so that at present very little information is available on the oxidation of human VLDL isolated from normolipidemic subjects. We have studied the oxidative modification of human LDL mediated by peroxyl radicals and transition metals like copper and iron salts. We have observed that the small amounts of ubiquinol-10 (QH2) associated with LDL provide an important and highly efficient antioxidant defense in the absence of QH2 and other suitable reductants, α-tocopherol (TOH, the quantitatively major lipid-soluble antioxidant in LDL) can act as a pro-oxidant. We have also reported that during the initial stages of radical-mediated oxidation of human blood plasma from fasted donors, lipoprotein lipid oxidation occurred preferentially in HDL rather than LDL, and we suggested that this was due to the apparent absence of QH2 from HDL. In the present study we measured the endogenous antioxidants associated with freshly isolated human VLDL from healthy subjects and compared its free radical-mediated oxidation with that of LDL and HDL. The results show that VLDL contains QH2 and that the oxidation kinetics of VLDL lipids is similar to that of LDL. Furthermore, competition experiments revealed that unlike HDL lipids, VLDL lipids are oxidized simultaneously with those of LDL.
Methods

Affi-Gel Blue Gel (50 to 100 mesh) was obtained from Bio-Rad, and tritoleate, trilinoleate, cholesteryl oleate, cholesteryl linoleate, and cholesteryl arachidonate were from Sigma Chemical Co. Ubiquinone-10 and all E)-lycopene were generous gifts from Mitsubishi Gas Chemicals and H. Koller (Hoffmann-La Roche), respectively. The OH2 standard was prepared from ubiquinone-10 by dithionite reduction14 and immediately used after its preparation. Lipid hydroperoxide standards were prepared from the corresponding purified lipids.19 Organic solvents of high-performance liquid chromatography (HPLC) quality and "nanopure" water were used throughout. All aqueous solutions and buffers were treated with Chelex-100 (Bio-Rad) to remove contaminating transition metals. All other chemicals used were the same as described19 and were of the highest purity available.

Plasma was prepared from blood obtained from nonfasted, healthy, normolipidemic subjects (25 through 40 years old) under the guidelines of and with approval from the local human ethics committee. The blood was heparinized immediately, and VLDL was isolated by three consecutive centrifugations as a result of the elimination of the contaminating triglyceride (TG)-rich lipoprotein fraction (TRL) using a benchtop ultracentrifuge (Beckman Instruments). Briefly, 5.1-mL polystyrene polyallomer tubes (Beckman) were filled with 3 mL, phosphate-buffered saline (PBS; 150 mmol/L NaCl and 50 mmol/L PO42-, pH 7.4) containing 0.1% EDTA and underlayered with 2 mL plasma, the density of which had been adjusted to d = 1.21 g/mL with solid KBr. The tubes were placed into a TLA-100.4 fixed-angle rotor (Beckman) and centrifuged at 15°C and 417 000g for 10 hours. The visible LDL band was withdrawn by appropriate piercing the tubes, and the LDL-obtained gel was filtered by using a PD-10 Sephadex G-25M column (Pharmacia LKB Biotechnology) and used for the VLDL/LDL oxidation-compartment experiments.

For VLDL isolation, the tubes were sliced at the top, and the triglyceride (TG)-rich lipoprotein (TRL)—containing top fraction was collected. Separation of TRL into chylomicrons and VLDL was achieved by using the method of Traber et al20 with minor modifications. The TRL-containing fraction was dispensed (0.75 mL) into 2-mL polystyrene polyallomer tubes and carefully overlayered with PBS of density d = 1.006 g/mL and containing 0.1% EDTA. The samples were centrifuged at 100 000 rpm for 5 minutes at 4°C by using a TLA-100.2 fixed-angle rotor (Beckman), the chylomicron-containing fraction was discarded, and the remaining solution was transferred to 2-mL polystyrene polyallomer tubes. The latter were then centrifuged for 25 minutes at 100 000 rpm using the TLA-100.2 rotor, the tubes were sliced, and the VLDL present at the top was collected for further experiments.

To remove small amounts of contaminating albumin, VLDL fractions (2 mL) were passed over an Affi-Gel Blue Gel column (25×15-mm diameter settled bed volume) using PBS as eluent, and the VLDL-containing fractions, which showed slight turbidity against a dark background, were collected. Affi-Gel Blue Gel—treated and untreated VLDL contained the same amounts of free cholesterol, cholesteryl ester (CE), or TOH. However, the protein content was lower in the treated samples (2 mL)0.26 to 0.53 mg protein/mL, corresponding to 50 to 100 nmol/L) was incubated in PBS at 37°C with aqueous solutions of the thermobital peroxyl radical generator 2,2′-azo-(2-amidinopropane hydrochloride) (AAPH). At various time intervals, 200-mL aliquots of the reaction solution were withdrawn, added to cold methanol (2 mL) containing 0.02% acetic acid, and extracted by vigorous shaking with cold hexane (10 mL). After centrifugation of the biphasic extracts, the supernatants were removed and evaporated to dryness, and the residue was redissolved in methanol/2-buty alcohol (1:1, vol/vol) and analyzed immediately for lipid-soluble antioxidants, TGs, and unoxidized CE and free cholesterol by HPLC methods12,23.
Lipids and Antioxidants in Human VLDL From Plasma of Six Healthy Subjects

<table>
<thead>
<tr>
<th>Subject, Sex</th>
<th>VLDL, nmol/L</th>
<th>Protein, mg/mL</th>
<th>TG, mmol/L (mg/dL)</th>
<th>Free Cholesterol, μmol/L (mpp)</th>
<th>Cholesterol Ester, μmol/L (mpp)</th>
<th>Coenzyme Q₁₀* μmol/L (mpp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>50.8</td>
<td>0.4†</td>
<td>1.25 (111)</td>
<td>139 (2736)</td>
<td>247 (4862)</td>
<td>0.30 (1.5)</td>
</tr>
<tr>
<td>M</td>
<td>67.1</td>
<td>n.d.</td>
<td>1.71 (151)</td>
<td>309 (4605)</td>
<td>664 (9896)</td>
<td>0.41 (6.4)</td>
</tr>
<tr>
<td>M</td>
<td>128</td>
<td>0.56</td>
<td>3.34 (296)</td>
<td>457 (3567)</td>
<td>1069 (8352)</td>
<td>0.31 (2.5)</td>
</tr>
<tr>
<td>F</td>
<td>7.0</td>
<td>0.02</td>
<td>0.18 (16)</td>
<td>20.6 (2943)</td>
<td>33 (4714)</td>
<td>0.02 (2.9)</td>
</tr>
<tr>
<td>M</td>
<td>30.7</td>
<td>0.16</td>
<td>0.78 (69)</td>
<td>100 (3257)</td>
<td>152 (4951)</td>
<td>0.07 (2.2)</td>
</tr>
<tr>
<td>M</td>
<td>75.7±14.82</td>
<td>0.4±0.03</td>
<td>2.0 (177)</td>
<td>311 (3988)</td>
<td>587 (7484)</td>
<td>0.55 (7.0)</td>
</tr>
</tbody>
</table>

n=5

VLDL indicates very-low-density lipoprotein; TG, triglycerides; and mpp, molecules per VLDL particle. Concentrations given refer to the VLDL preparations obtained. The concentration of VLDL was calculated from both protein and triglyceride determinations of the lipoprotein, assuming a mean molecular weight for VLDL of 4.5×10⁵ D and mean relative molecular masses of 50% and 10% for VLDL triglycerides and proteins, respectively. Both calculations gave comparable molar concentrations of VLDL. Triglyceride concentrations were calculated as triolein equivalent. The bottom row represents mean±SD values of one individual determined from five independent isolations. Other results are mean (SD).

Coenzyme Q₁₀ is ubiquinone-10+ubiquinol-10.

†VLDL sample not passed over Affi-Gel Blue Gel column.

Separately by using the methods described above. The concentration of each lipoprotein was determined before they were recombined at a ratio resembling that of these two lipoproteins in normal, nonfasted human plasma, i.e., about 37 LDL particles for 1 VLDL particle. The lipoprotein mixture (4 mL) (LDL 0.56 mg protein/mL or 1.12 mmol/L, VLDL 0.39 mg protein/mL or 0.074 mmol/L) was incubated under air at 37°C with AAPH (1 mmol/L) and 400-mL aliquots were removed at different time points and stored on ice. Placing the samples on ice virtually halted the oxidation. Each aliquot was then recombined at a ratio resembling that of these two lipoproteins for independent isolations. Other results are mean (SD).

**Results**

The Table shows the concentrations of lipid-soluble antioxidants, TGs, cholesterol, and CEs in VLDL prepared at different concentrations from plasma obtained from six different normolipidemic individuals. Also shown are the mean±SD values of VLDL lipids and antioxidants of one individual determined from five independent isolations. The relative concentrations of individual lipids and antioxidants varied substantially among the six subjects, consistent with similar large variations reported for human LDL. Although TOH was quantitatively the major antioxidant, with its levels and those of β-carotene detected in our study being in good agreement with literature data. There is little information available at present on the concentration of QH₂ in human VLDL preparations. The Table presents the sum of the reduced (QH₂) and oxidized (ubiquinone-10) forms of coenzyme Q₁₀. Although in most VLDL preparations the redox status of coenzyme Q₁₀ favored ubiquinone-10, this was likely the result of inadvertent auto-oxidation of QH₂ (to ubiquinone-10) during the lengthy isolation procedure. This is supported by the fact that with increasing isolation speed and care to prevent auto-oxidation (by performing as many steps of the isolation procedure as possible under argon), the redox status of coenzyme Q₁₀ in the resulting VLDL increased to a QH₂/ubiquinone-10 ratio of up to 3:1. A redox ratio in favor of the reduced form is consistent with our observation on VLDL-containing TRL isolated rapidly from human plasma. We conclude from both these results that VLDL, like LDL, contains small but significant amounts of QH₂.

To examine VLDL oxidation and antioxidation, we exposed freshly prepared VLDL (0.29 mg protein/mL, corresponding to 65 nmol/L) to a constant flux of aqueous peroxyl radicals produced by the thermal decomposition of the azo compound AAPH. The radical initiator was used at 0.5 mmol/L to give a calculated rate of peroxyl radical generation (Rg) of 5.5×10⁻¹⁰ mol/L·s⁻¹ (Rg=1.1×10⁻⁴ [AAPH], as described for aqueous protein-containing solutions). Determination of the effective rate of peroxidation initiation (Rg) in VLDL (Rg=−2d[TTOH]/dt) revealed a value of 1.2×10⁻¹⁰ mol/L·s⁻¹, suggesting that most of the radicals generated in the aqueous phase by AAPH reacted with each other rather than with components of VLDL. A low efficacy of lipid oxidation initiation by AAPH has been observed with LDL. Exposure of VLDL to such radical flux resulted in immediate disappearance of QH₂, with approximately 80% being consumed rapidly and linearly within the first hour of incubation (Fig 1). In contrast, a corresponding control incubation of VLDL in the absence of AAPH resulted in a much slower rate of (auto)oxidation of QH₂, with more than 20% of this antioxidant remaining even after 5 hours of incubation (not shown). Also, peroxyl radical-induced consumption of TOH (Fig 1), lycopene, and β-carotene (not shown) was very slow (d[TTOH]/dt=5.7×10⁻¹¹ mol/L·s⁻¹), and even after 5 hours of incubation of VLDL with 0.5 mmol/L AAPH at 37°C more than 90% of these antioxidants remained present.

Lipid hydroperoxides were detected neither in freshly isolated VLDL nor in the lipoprotein during the first 30 minutes of the oxidation, suggesting that the endogenous antioxidants associated with this lipoprotein efficiently protected its lipids from oxidative damage. Only small amounts of lipid hydroperoxides were detectable
in oxidizing VLDL as long as more than 20% of the original QH₂ was present, whereas TGOOHs and CEOOHs (see below) were formed at markedly increased rates (Fig 1). Thus, VLDL TGs peroxidized at a rate (Rₚ) of 2.6×10⁻⁹ mol/L • s⁻¹ following consumption of most QH₂ and while normal levels of TOH were present. The observed peroxidation rate corresponded to an apparent chain length (κ, ν=κRₚ/Rₒ) of 17.3, demonstrating that the AAPH-induced oxidation of VLDL TGs can proceed in a chain reaction of substantial length during the TOH-inhibited phase. Throughout oxidation, the TGOOH/CEOOH ratio remained close to the VLDL TG/VLDL CE ratio, ie, approximately 5:1. This indicates that the fractional peroxidation rates of the two core lipids were similar. Lipid hydroperoxides were not detected in VLDL incubated for up to 5 hours in the absence of AAPH (not shown).

Fig 2 (top) shows a typical chromatogram of a VLDL lipid extract monitored at 210 nm, with the peaks numbered 1 through 3 representing the major (unoxidized) TG groups of complex fatty acid composition. TGs constitute more than 60% of the total lipids in VLDL, with palmitic, oleic, and linoleic acids being the major fatty acid esters.²² Because of their complexity in composition we did not attempt to separate all individual species of VLDL TGs and resulting TGOOHs. Under identical chromatographic conditions, authentic trilinoleate and trioleate eluted with retention times of 8.8 and 12.8 minutes, respectively (not shown). Fig 2 (bottom) shows a typical chromatogram of an organic lipid extract prepared from AAPH-oxidized VLDL with Ch18:2, the major hydroperoxide of the CE of the lipoprotein, eluting at around 35 minutes, as identified by coelution with an authentic standard. Lipid hydroperoxides were verified as such by sodium borohydride treatment of VLDL extracts, which led to a complete disappearance of the hydroperoxides of TGs and cholesterol esters (dotted line).
individual peaks (chromatograms not shown) with the lipid-soluble radical generator AMVN in methanol/t-butyl alcohol (1:1, vol/vol) led to the series of chemiluminescence-positive peaks 1 through 3.

The oxidation of lipids in isolated LDL exposed to either oxygen-saturated buffer,21 low concentrations of transition metals,32 or a mild radical flux30 proceeds in different stages, which may be divided into (1) a strongly inhibited phase (during which QH₂ is consumed), (2) a TOH-inhibited phase (during which TOH is consumed), and (3) a TOH-uninhibited phase (following consumption of TOH and other antioxidants). While not observed during the present studies, an interesting feature of LDL oxidation studies is that the rate of lipid oxidation during the TOH-inhibited phase is comparable to and in some cases even higher than that during the TOH-uninhibited period.30,32 We have also observed such kinetic behavior of lipid peroxidation during radical-mediated LDL oxidation and explained it on experimental and theoretical grounds by postulating that TOH acts as both a phase- and peroxidation chain-transfer agent.16.18 To examine whether oxidation of VLDL lipids proceeded with similar kinetics to those of LDL, we exposed the former to a higher concentration of AAPH (16 mmol/L) and examined R₈ during the TOH-inhibited and TOH-uninhibited periods. As expected, such treatment resulted in very rapid consumption of QH₂ and TOH disappeared from both LDL and VLDL (Fig 4) and then recombined at an LDL/VLDL molar ratio of 15 (LDL 0.56 mg protein/mL or 1.12 mmol/L, VLDL 0.39 mg protein/mL or 0.074 mmol/L). Oxidation of this lipoprotein mixture was obtained by incubation at 37°C in the presence of 3 mmol/L 2,2'-azobis(2-amino-2-aminopropane hydrochloride). At the times indicated, samples were withdrawn, and the lipoproteins were reisolated and analyzed for ubiquinol-10 (solid squares), α-tocopherol (solid circles), the hydroperoxides of cholesteryl esters (open squares with crossed lines), triglycerides (open squares with diagonal lines), and phosphatidylcholine hydroperoxide (open squares). The initial concentrations of ubiquinol-10 (open squares) and 7.9 μmol/L, respectively. The results shown are typical of three independent experiments performed with LDL isolated from different donors, with the initial concentrations of antioxidants within the range shown in the Table.

Discussion

VLDL and LDL differ greatly in particle size, a fact reflected in the different number of lipid and antioxidant molecules in the two lipoprotein particles. While
we observed that the free cholesterol levels in VLDL varied among different individuals, there were on average close to 4000 cholesterol molecules per lipoprotein particle. This value is about half of that in Lenter but agrees well with the data reported by Winocour et al for large VLDL particles. More interesting in the context of lipoprotein oxidation, VLDL contained significant amounts of OH. Dietary coenzyme Q\textsubscript{10} reaches the liver, from where it and newly synthesized coenzyme Q\textsubscript{9} are secreted within VLDL; once present in the circulation, coenzyme Q\textsubscript{10} is distributed among other lipoproteins and may be taken up slowly by at least some tissues. Our observation that the ratio of TOH/ubiquinone-10+OH\textsubscript{2} was only somewhat higher in VLDL (Table) than LDL is consistent with these reports and suggests that these lipoproteins may, like TOH, transport some coenzyme Q\textsubscript{10} to extrahepatic tissues.

Our findings indicate further that coenzyme Q\textsubscript{10} in human VLDL is present largely in its reduced, antioxidant form. Indeed, VLDL Q\textsubscript{H2} is capable of efficiently protecting VLDL lipids against radical-mediated oxidative damage. The antioxidant action of Q\textsubscript{H2} in VLDL appears to be similar to that in LDL, i.e., most likely mediated by reduction of \( \alpha \)-tocopheroxyl radical within the lipoprotein. Direct radical scavenging by OH\textsubscript{2} is unlikely considering its similar (to TOH) reactivity toward peroxyl radicals and that there are at least 30 molecules of TOH for every molecule of QH\textsubscript{2} in VLDL. Also, regeneration of TOH from \( \alpha \)-tocopheroxyl radicals alone cannot explain the high antioxidant efficacy of QH\textsubscript{2} as in this case the antioxidant defense of VLDL would have to rely on TOH alone. The latter notion, however, is incompatible with the observed sharp increase in the rate of lipid peroxidation during the early stages of VLDL oxidation, when the concentrations of TOH did not change substantially. Considering the similarities between the kinetics of lipid peroxidation in VLDL (this study) and LDL, we conclude that the recently proposed mechanism of tocopherol-mediated peroxidation is also likely to be operative during peroxyl-mediated and other radical-mediated VLDL oxidation. According to this mechanism, \( \alpha \)-tocopherol radicals inside VLDL (or LDL) cannot readily leave the particle and, unless eliminated, eg, through reduction by OH\textsubscript{2} (or ascorbate), are forced to react with polyunsaturated lipids, thereby acting as an oxidation chain-carrying agent.

Oxidation competition experiments using physiological concentrations and molar ratios of LDL and VLDL revealed that the frequency with which the randomly produced radicals initiated lipid peroxidation in LDL was some 2.7-fold greater than in VLDL. This value is comparable to the ratio of the total surfaces of the two lipoproteins (ie, \( 13.5 \times 1810 \text{ nm}^2 = 24435 \text{ nm}^2 \) for LDL and \( 1 \times 9503 \text{ nm}^2 = 9503 \text{ nm}^2 \) for VLDL; \( \text{surface}_{\text{LDL}}/\text{surface}_{\text{VLDL}} = 2.6 \)). This indicates that the aqueous peroxyl radicals do not discriminate between the two lipoprotein particles, so that neither one of them is oxidized preferentially. Since the chromanol moiety of TOH is by far the most reactive group a peroxyl radical can encounter on the surface of lipoproteins, these results suggest that the TOH/surface ratio in VLDL is similar to that in LDL. Once peroxidation is initiated within the lipoprotein particles, the resulting effective oxidation chain length is more than twofold higher in VLDL than LDL (Fig 4). Assuming that chain terminating reactions are mostly due to interactions between the single (trapped) radical within the lipoprotein particle and AAPH-derived radicals in the aqueous phase, a longer oxidation chain length in VLDL than LDL may be rationalized by the larger volume/surface ratio of the former (ie, 9.2 and 4.0 for VLDL and LDL, respectively). Thus, as the volume/surface ratio increases, the probability for a mobile tocopheroxyl radical residing in a lipoprotein to undergo oxidation chain termination reactions on the particle surface decreases. By analogy, in HDL, with its low volume/surface ratio, the oxidation chain length should be relatively small, as indeed has been observed.

In conclusion, our studies suggest that radical-mediated lipid oxidation in VLDL proceeds via similar mechanisms as in LDL. Therefore, efficient LDL antioxidants are likely to be effective protective agents for VLDL.

Acknowledgments

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References

1. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witzum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. \( N \text{ Engl J Med.} \ 1989;320:915-924. \)
4. Witzum JL, Steinberg D. Role of oxidized low density lipoprotein in atherosclerosis. \( J \text{ Clin Invest.} \ 1991;88:1785-1792. \)
11. Sparrow CP, Parthasarathy S, Steinberg D. A macrophage receptor that recognizes oxidized low density lipoprotein but not acetylated low density lipoprotein. \( J \text{ Biol Chem.} \ 1989;264:2599-2604. \)
12. Stocker R, Bowry WW, Frei B. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does \( \alpha \)-tocopherol. \( Proc \text{ Nail Acad Sci U S A.} \ 1991;88:1646-1650. \)
13. Mohr D, Bowry WW, Stocker R. Dietary supplementation with coenzyme Q10 results in increased levels of ubiquinol-10 within circulating lipoproteins and increased resistance of human low
27. Elmerberger PG, Kalén A, Brunk UT, Dallner G. Discharge of newly-synthesized dolichol and ubiquinone with lipoproteins to rat liver perfusate and to the bile. Lipids. 1989;24:919-930.
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