Age-related decrease of dehydroepiandrosterone concentrations in low density lipoproteins and its role in the susceptibility of low density lipoproteins to lipid peroxidation

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Abstract **The incidence of atherosclerosis and related diseases increases with age. The aging process may enhance lipoprotein modification, which leads to an increase in the susceptibility of low density lipoprotein (LDL) and high density lipoprotein (HDL) to oxidation. Dehydroepiandrosterone (DHEA), the most abundant steroid hormone in humans, has been shown to have antiatherogenic effects. This hormone also decreases dramatically with age. In the present study, we were interested in determining the presence of DHEA/DHEAS (dehydroepiandrosterone sulfate) and changes in their concentrations in HDL and LDL lipoproteins with age. Moreover, we studied the susceptibility of LDL to oxidation with age in the presence or absence of vitamin E or DHEA. We demonstrated that vitamin E is unable to restore the decreased resistance to oxidation of LDL from elderly subjects to that of LDL obtained from young subjects. Furthermore, our results provide evidence that DHEA is an integral part of LDL and HDL and disappears to almost nondetectable levels during aging. The DHEA incorporated into the LDL from elderly subjects increased LDL resistance to oxidation in a concentration-dependent manner. The increased resistance provided by DHEA was higher than that with vitamin E. DHEA seems to act either by protecting vitamin E from disappearance from LDL under oxidation or by scavenging directly the free radicals produced during the oxidative process. Our results suggest that DHEA exerts an antioxidative effect on LDL, which could have antiatherogenic consequences. Careful clinical trials of DHEA replacement should determine whether this ex vivo effect could be translated into any measurable antiatherogenic (cardioprotective) action.**—A. Khalil, J-P. Fortin, J-G. LeHoux, and T. Fülöp. **Age-related decrease of dehydroepiandrosterone concentrations in low density lipoproteins and its role in the susceptibility of low density lipoproteins to lipid peroxidation.** ***J. Lipid Res.*** **2000.** 41: **1552–1561.**

Supplementary key words aging • atherosclerosis • LDL • HDL • free radicals • DHEA • vitamin E

Low density lipoproteins (LDL) are considered as the major atherogenic lipoproteins (1). Oxidative modifications of LDL enhance their atherogenecity. Oxidation of LDL, in vivo, may be initiated by oxygen free radicals originating from several cellular types, such as macrophages, or endothelial or smooth muscle cells (2–4). Unlike native LDL, oxidized LDL (ox-LDL) are rapidly taken up by scavenger receptors of monocytes/macrophages as a result of derivatization of apolipoprotein B by products of lipid peroxidation. The uptake of ox-LDL by these scavenger receptors prevents downregulation of cholesterol synthesis, which thus leads to the development of foam cells presenting the pathophysiological basis of fatty streak formation (5, 6).

The incidence of atherosclerosis and its related diseases [stroke and coronary heart diseases (CHD)] increases with aging (7–12). The aging process may enhance lipoprotein modification (12), which leads to the increase in the susceptibility of LDL and high density lipoprotein (HDL) to oxidation (13, 14). Moreover, aging is associated with several important biochemical modifications in the arterial wall and in lipoproteins (15, 16) as well as in hormonal status such as dehydroepiandrosterone (17, 18) and estrogen (19), which may be implicated in the increased incidence of CHD.

Dehydroepiandrosterone (DHEA) is quantitatively the most abundant steroid hormone biosynthesized in humans, with a wide variety of physiological effects (20, 21). This hormone decreases dramatically with age (22, 23). For in-

Abbreviations: CHD, coronary heart disease; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; EDTA, ethylenediaminetetraacetic acid; EGTA, ethyleneglycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; LDL/HDL, low/high density lipoproteins; MDA, malondialdehyde; ox-LDL, oxidized LDL; TBARS, thiobarbituric acid-reactive substances.

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stance, in the sera of 80-year-old subjects, the DHEA concentration is only 10% of that found in the sera of 25-year-old subjects (24). DHEA has numerous biological and biochemical effects (21). In animal models, DHEA has been shown to have a beneficial effect on diabetes, obesity, cancer, stress, and viral and bacterial infection (25–30). Administration of DHEA in a hypercholesterolemic rabbit model of heterotopic cardiac transplantation significantly retards the progression of accelerated atherosclerosis in both the transplanted heart and in the native heart (31). The age-related fall of DHEA in men prompted the thesis that low levels might be associated with diseases of aging such as atherosclerosis (32, 33). Epidemiological studies showed an antiatherogenic action of DHEA or its sulfated form (DHEAS) (32, 34). However, it should be noted that other studies failed to demonstrate beneficial effects of DHEA on administration to humans or animals, either in relation to cardiovascular diseases or other biological or metabolic effects (35–39). Nevertheless, despite the observed biological effects of DHEA little is known about its mechanism of action. In vitro, DHEA has been demonstrated to have an antioxidative action against lipid peroxidation in tissue (40, 41). It has been reported by us and other groups that DHEA protects LDL from oxidation induced either by oxygen free radicals, produced by γ radiolysis, or metal ions (42, 43). This protective effect of DHEA was explained by vitamin E sparing as well as by preventing the change of both enzymatic and nonenzymatic antioxidants induced by oxidative stress (41, 42, 44).

In humans, 90% of the circulating DHEA is bound to albumin and a further 3% circulates in association with sex hormone-binding globulin (45). However, just as for HDL (46), DHEA esters may also incorporate into the LDL fraction. The presence of esterified DHEA in LDL could stabilize lipids contained in these particles against oxidation (42). The decrease of DHEA content in LDL with aging may be implicated in the increase of their susceptibility to oxidation (14, 42).

In this study, we were interested in determining the presence of DHEA/DHEAS and any concentration changes in HDL and LDL with age. Moreover, we studied the susceptibility of LDL from elderly subjects to oxidation in the presence or absence of DHEA or vitamin E.

MATERIALS AND METHODS

Acetic acid, sulfuric acid, *n*-butanol, sodium phosphate, thiobarbituric acid, methanol, hexane, monobasic potassium phosphate, potassium iodide, and sodium azide were purchased from Fisher Scientific (Montréal, QC, Canada). D-α-Tocopherol, DL-α-tocopherol, DHEA (5-androsten-3β-ol-17-one), 1,1,3,3-tetraethoxypropane, ethyleneglycol-bis(β-aminoethyl ether)-*N,N,N′, N′*-tetraacetic acid (EGTA), benzalkonium chloride, ammonium molybdate, and butylated hydroxy toluene (BHT) were obtained from Sigma (St. Louis, MO), and Triton X-100 was from ICN Biochemicals (Aurora, OH). Dialysis bags were purchased from Spectrum Medical Industries (Houston, TX) and [4-^{14}C]DHEA was purchased from NEN-DuPont Canada (Merkham, ON, Canada). ^{125}I-labeled DHEA-SO_4 (Coat-A-Count DHEA sulfate) was from Diagnostic Products (Los Angeles, CA) and ^{125}I-labeled DHEA was from Diagnostic Systems Laboratories (Webster, TX).

LDL isolation

LDL were isolated from human plasma collected in EDTA (0.4 g/liter) obtained from 10 healthy young subjects (20–25 years; mean age, 22.4 ± 1.95 years), 10 middle-aged subjects (30–45 years; mean age, 39.3 ± 4.03 years), and 10 elderly subjects (65 years and over; mean age, 70.4 ± 4.42 years), according to the method of Sattler, Mohr, and Stocker (47). All subjects were males. Isolated LDL were dialyzed overnight at 4°C with 10^{-2} M sodium phosphate buffer (pH 7) with argon bubbling at a rate of 20 ml/min through the dialysis solution (4 liters, changed twice) to ensure anaerobic conditions. Concentrations of LDL solutions were given in terms of total protein concentrations (100 μg/ml). Proteins were measured by commercial assay (Pierce, Rockford, IL).

The study was approved by the Medical Ethics Committee of the Sherbrooke Geriatric University Institute and all subjects gave written informed consent.

LDL oxidation by γ radiolysis

γ irradiations were carried out with a ^{60}Co Gamma Cell 220 (Atomic Energy of Canada, Mississisauga, ON, Canada) as previously described (48). The dose rate was 0.13 Gy/sec as determined by Fricke and Morse dosimetry (49). Total radiation doses were varied from 0 to 300 Gy. Briefly, LDL were irradiated in oxygenated aqueous solutions containing 10^{-2} M sodium phosphate buffer at pH 7, in the presence or absence of vitamin E or DHEA. Vitamin E and DHEA were dissolved in ethanol and the final concentration of ethanol in the irradiated solutions was 34 mM.

In our experimental system, irradiation of aqueous solutions with ionizing radiation (γ rays of ^{60}Co in our case) leads to the formation of three major initial free radicals: hydroxyl radicals ($^{\bullet}OH$), hydrated electrons (e^{-}_{aq}), and hydrogen atoms ($H^{\bullet}$) (50). In the presence of oxygen, e^{-}_{aq} and $H^{\bullet}$ are transformed quantitatively to superoxide anions ($O_2^{\bullet -}$) and perhydroxyl radicals ($HO_2^{\bullet}$), respectively. $HO_2^{\bullet}$ is the acidic form of $O_2^{\bullet -}$ (pK_a 5.7) (51). Under our conditions, the concentration of ethanol, an efficient scavenger of the $^{\bullet}OH$, was 34 mM, suggesting that most of the $^{\bullet}OH$ radicals react with ethanol to form the α-hydroxyethyl radical $CH_3{}^{\bullet}CHOH$ (denoted $R^{\bullet}$) (50, 52). The latter species are then transformed into peroxyl radicals, $RO_2^{\bullet}$. Thus, the reactive oxygen species generated by γ irradiation in our experiments are $O_2^{\bullet -}$, $RO_2^{\bullet}$, and $^{\bullet}OH$.

LDL enrichment with vitamin E or DHEA

Plasma of each donor was supplemented with vitamin E or DHEA, for 4 and 24 h at 37°C under gentle stirring and under a N_2 atmosphere. Final concentrations were as follows: vitamin E, 100 μM; DHEA, 0.1 and 1 μM; and ethanol, 34 mM.

Measurement of DHEA and DHEAS

DHEA and DHEAS in serum and in LDL/HDL were measured by radioimmunoassay (RIA) (53) in our endocrinology clinical laboratory, according to the supplier recommendations.

DHEA and DHEAS extraction

Lipoproteins were precipitated in 5 volumes of ethanol–acetone 1:1 and stored overnight at −20°C. Samples were then centrifuged and supernatant was dried and suspended in the zero calibrator provided with the dosage kit.

DHEA measurement.

We added 500 μl of ^{125}I-labeled DHEA and 100 μl of DHEA antiserum to 100 μl of sample. The tubes were then incubated at 37°C for 1 h. One milliliter of precipitating reagent was added and the tubes were incubated at room temperature for 15 min.

After centrifugation (1,500 *g*, 20 min) all tubes were decanted and the radioactivity was then measured in the pellet. DHEAS measurements were performed with ^{125}I-labeled DHEA-SO_4 and Coat-A-Count DHEA sulfate (Diagnostic Products). Briefly, 50-μl samples were placed in DHEA-SO_4 Ab-coated tubes and 1.0 ml of the tracer, ^{125}I-labeled DHEA-SO_4, was dispensed into the tubes immediately. The tubes were then incubated for 30 min in a water bath (at 37°C). The contents of all tubes were decanted, and the tubes were allowed to drain for 2 or 3 min. The tubes were then tapped sharply on absorbent paper to shake off all residual droplets. The radioactivity was measured in a γ counter.

Data for DHEA and DHEAS are presented as counts per minute (cpm), and their concentrations were calculated from a log-log calibration curve.

Measurement of LDL oxidation

LDL oxidation induced by free radicals produced by γ radiolysis was monitored as follows.

Conjugated diene formation. LDL (100 μg/ml) irradiated alone or after incorporation of DHEA (either 0.1 and 1 μM) was continuously monitored at 234 nm to detect the formation of conjugated dienes as previously described (54).

Lipid hydroperoxide formation. Lipid hydroperoxide formation during LDL oxidation was detected according to the method of el-Saadani et al. (55). The principle of this assay is based on the oxidative capacity of lipid peroxides to convert iodide to iodine, which can be measured spectrophotometrically at 365 nm. The amount of lipid peroxide can be calculated with an extinction coefficient of $\varepsilon = 2.46 \times 10^4$ mol^{-1} cm^{-1}. The working reagent consists of 200 mM monobasic potassium phosphate (pH 6.2), 120 mM potassium iodide, 0.15 mM sodium azide, Triton X-100 (2.0 g/liter), benzalkonium chloride (0.1 g/liter), 0.01 mM ammonium molybdate, 0.02 mM BHT, and 0.024 mM EGTA. To detect lipid hydroperoxides formed, sample (100 μl) was added to 1 ml of working reagent and incubated in the dark at room temperature for 30 min. The samples were then read with a U-300 spectrophotometer (Hitachi, Tokyo, Japan) at wavelength 365 nm.

Thiobarbituric acid-reactive substance formation. Thiobarbituric acid-reactive substance (TBARS) formation was assayed as described by Yagi (56), but without precipitation with phosphotungstic acid (54). The coefficient of variation of this assay is less than 10%. TBARS concentrations were calculated as malondialdehyde (MDA) equivalents, using the MDA standard curve. MDA was generated by the hydrolysis of 1,1,3,3-tetraethoxypropane. DHEA (0.1 and 1 μM) had no effect on the standard curve.

Vitamin E measurement. Endogenous vitamin E in LDL was assayed as α-tocopherol, before and after irradiation, by reversed-phase high-performance liquid chromatography (HPLC), with UV detection at 292 nm and electrochemical detection as already described (57, 58). α-Tocopherol was assayed on a Sephasil peptide column (C_{18} 5μ ST 4.6/250) (Pharmacia Biotech, Piscataway, NJ). The elution phase (1.2 ml/min) was a solvent mixture of methanol–ethanol–isopropanol 88:24:10 (v/v/v) and lithium perchlorate (20 μM).

Study of [4-^{14}C]DHEA incorporation in separated LDL fractions

Serum was incubated for 24 h with 5.5 μCi of [4-^{14}C]DHEA, followed by a simultaneous separation of very low density (VLDL), low density (LDL), and high density (HDL) lipoproteins according to the method of Sattler, Mohr, and Stocker (47). The remaining fraction (bottom) was also recovered for [4-^{14}C]DHEA analysis. The [4-^{14}C]DHEA-labeled lipoprotein fractions were counted in a β-scintillation counter (1600 TR liquid scintillation analyzer; Packard, Downers Grove, IL) in vials containing 4 ml of scintillation fluid, and the activity of each fraction was compared with initial activity in serum and expressed as a percentage.

Determination of the oxidation resistance (lag phase) of LDL

The resistance of all LDL samples against γ radiolysis-mediated oxidation was measured photometrically by following the change in the absorption of the conjugated dienes as previously described (59). Lag phases for TBARS and lipid peroxides were also used to determine the LDL resistance to oxidation.

Initial radiation dose yields (G) were determined as the initial slopes of the curves and as the slope of the linear part of the curves. Results were expressed as the decrease in α-tocopherol and the amount of TBARS and conjugated dienes formed per unit of energy (joules) absorbed, respectively. These yields reflected the rates of disappearance of α-tocopherol and formation of peroxidation products as a result of the action of the oxygen generated free radicals produced by γ radiolysis at steady state concentration.

Statistical analysis

Results are presented as pooled data from 10 independent experiments (means ± SD), done in duplicate. Mean values were compared by the Student's *t*-test, for detection of significant differences. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Increased susceptibility of LDL to oxidation with aging and the protective effect of vitamin E

Initial experiments were carried out to determine the effect of aging on the susceptibility of LDL to oxidation and the role of vitamin E. To determine susceptibility to oxidation, LDL obtained from healthy young (20–25 years) or elderly subjects (65 years and over) were subjected to the action of oxygen free radicals ($O_2^{-\bullet}$, $RO_2^{\bullet}$, and $^{\bullet}OH$). Conjugated diene formation was measured at each radiation dose (0 to 200 Gy) (**Fig. 1**). We observed that the onset of conjugated diene formation in LDL from elderly subjects takes place at lower doses than that for young subjects (Fig. 1). The onset or the lag phase, before appreciable peroxidation, was measured from the intercept of the initiation and the propagation phases of peroxidation. Thereby, the lag phase for conjugated diene formation of irradiated LDL was higher in young compared with elderly subjects (**Table 1**). At high radiation doses, the formation of conjugated dienes reached a plateau, which was higher for LDL from elderly subjects.

Enrichment of LDL, from young and elderly subjects, by vitamin E induced a prolongation of the lag phase. The vitamin E reduces oxidation in LDL obtained from both young and elderly subjects ($P < 0.01$). The propagation of the lipid peroxidation is also decreased in the LDL obtained from the two age groups (Table 1). However, differences in the oxidizability of LDL according to age persist (see Table 1). Consequently, the differences in vitamin E concentrations alone could not account for the difference in the susceptibility of LDL to oxidation observed with increasing age.

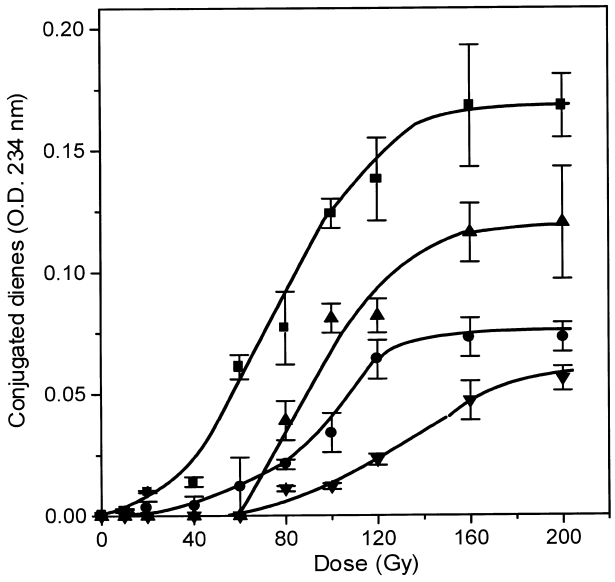

Fig. 1. Conjugated diene formation in native or vitamin E-enriched LDL as a function of irradiation dose. LDL were obtained from young or elderly subjects. Free radicals were generated by γ radiolysis of water (ethanol, 34 mM). The concentration of LDL was 100 μg/ml in aerated aqueous solutions containing 10^{-2} M sodium phosphate buffer at pH 7. Conjugated diene formation was followed by differential absorption at 234 nm ($\varepsilon_{234\ nm}$ = 27,000 M^{-1} cm^{-1}). LDL from elderly subjects (solid squares); elderly subject LDL enriched with vitamin E (solid triangles); LDL from young subjects (solid circles); young subject LDL enriched with vitamin E (solid inverted triangles). Results are presented as pooled data from 10 independent experiments performed in duplicate (means ± SD).

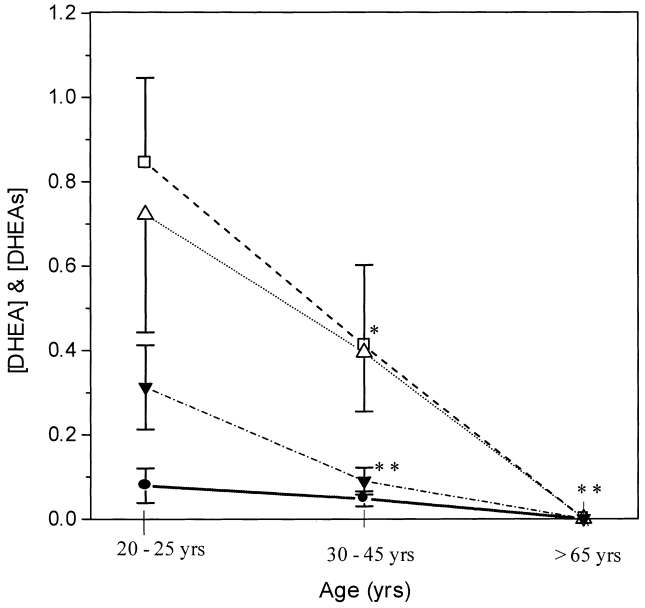

Fig. 2. DHEA and DHEAS concentrations in LDL and HDL as a function of age. DHEA and DHEAS were analyzed by radioimmunoassay. LDL and HDL were isolated from human plasma collected in EDTA (0.4 g/liter) obtained from 10 young (20–25 years), 10 middle-aged (30–45 years), and 10 elderly healthy subjects (65 years and over). LDL and HDL were adjusted to a concentration of 500 μg/ml, given in terms of total protein concentrations. Values are expressed as means ± SD. * $P < 0.05$ or ** $P < 0.02$ compared with young subjects. LDL: DHEA concentration (nanomolar; open squares), DHEAS concentration (micromolar; solid circles); HDL: DHEA concentration (nanomolar; open triangles), DHEAS concentration (micromolar; solid inverted triangles).

Measurement of DHEA/DHEAS concentrations in LDL and HDL as a function of age

Figure 2 displays the change in DHEA and DHEAS concentrations in LDL and HDL as a function of age. Three groups of age were studied: young (20–25 years), middle-aged (30–45 years), and elderly subjects (>65 years). DHEA and DHEAS were analyzed in LDL and HDL (500 μg/ml) immediately after separation by RIA. Our results show that DHEA and DHEAS are naturally associated with LDL and HDL and their concentrations decreased dramatically with age ($P < 0.02$). For the elderly group (>65 years), DHEA and DHEAS concentrations were so low that they were almost undetectable. Incubation of DHEA with plasma induces an increase in its concentration within LDL and HDL (results not shown).

TABLE 1. Susceptibility of LDL from young and elderly subjects to oxidation promoted by $^{\cdot}OH$, $O_2^{-\cdot}$ and $RO_2^{\cdot}$ free radicals induced by water radiolysis and the protective effect of LDL enrichment with vitamin E

	Lag Phase		Propagation Phase		Termination Phase	
	−Vit. E	+Vit. E	−Vit. E	+Vit. E	−Vit. E	+Vit. E
	Gy		*μM J*$^{-1}$		*μM*	
LDLy	35	70	0.46	0.21	0.27	0.20[a]
LDLe	20	60	0.71	0.61	0.62	0.43[a]

LDLy and LDLe, LDL from young and elderly subjects, respectively; −vit. E, without vitamin E; +vit. E, LDL enriched with vitamin E.
[a] $P < 0.01$ compared with LDL oxidized without vitamin E.

Susceptibility of DHEA-enriched LDL from elderly subjects to oxidation

In the second part of our studies, we were interested in investigating the effect of DHEA on the susceptibility to oxidation of LDL from elderly subjects (65 years and over). DHEA (dissolved in ethanol) was added to plasma and incubated for 4 h at 37°C under N_2 atmosphere. Incubation for a longer time, 24 h, gave the same result as for 4 h. LDL were separated as described in Materials and Methods and DHEA was analyzed by RIA. Approximately 30% of the DHEA added to plasma was found within LDL, indicating that the hydrophobicity of DHEA might enhance its association with LDL. Less than 10% was associated with the HDL, and the remainder of the DHEA was found in VLDL and in plasma delipidated proteins (bottom of the tube).

LDL oxidation was followed by conjugated diene, lipid hydroperoxide, and TBARS formation as well as by vitamin E disappearance. Oxygen free radicals were produced quantitatively by the γ radiolysis of water. Two concentrations of DHEA (0.1 and 1 μM) were used and LDL without DHEA enrichment was used as a control. The concentration of DHEA, 0.1 μM, is three times higher than its concentration in plasma for young subjects. The effect of eth-

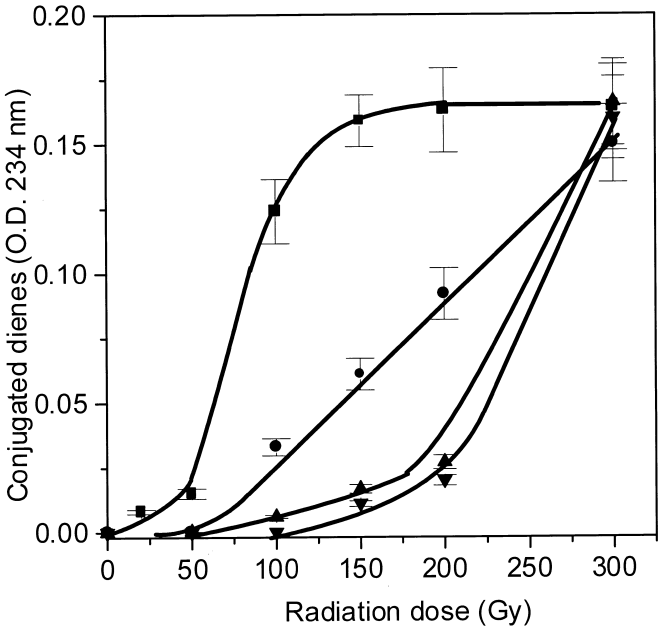

Fig. 3. Conjugated diene formation in LDL from elderly subjects as a function of radiation dose. LDL were enriched with DHEA by direct supplementation of DHEA to plasma before LDL separation. Free radicals were generated by the γ radiolysis of water (34 mM ethanol). The concentration of LDL was 100 μg/ml in aerated aqueous solutions containing 10^{-2} M sodium phosphate buffer at pH 7. Conjugated diene formation was monitored by differential absorption at 234 nm ($\varepsilon_{234\,nm}$ = 27,000 M^{-1} cm^{-1}. LDL alone (solid squares); LDL plus 34 mM ethanol (solid circles); LDL enriched with 0.1 μM DHEA (solid triangles); LDL enriched with 1 μM DHEA (solid inverted triangles). Results are presented as pooled data from 10 independent experiments performed in duplicate (means ± SD).

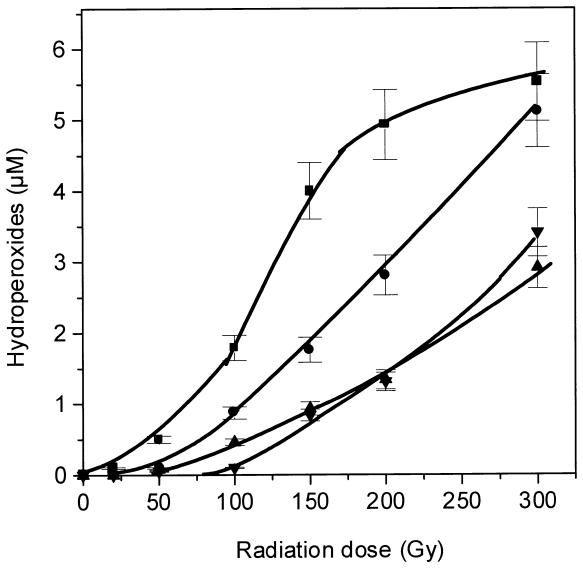

Fig. 4. Hydroperoxide formation in LDL from elderly subjects as a function of radiation dose. LDL were enriched with DHEA by direct supplementation of DHEA to plasma before LDL separation. Free radicals were generated by the γ radiolysis of water (34 mM ethanol). The concentration of LDL was 100 μg/ml in aerated aqueous solutions containing 10^{-2} M sodium phosphate buffer at pH 7. LDL alone (solid squares); LDL plus 34 mM ethanol (solid circles); LDL enriched with 0.1 μM DHEA (solid triangles); LDL enriched with 1 μM DHEA (solid inverted triangles). Results are presented as pooled data from 10 independent experiments performed in duplicate (means ± SD).

anol, used here to dissolve DHEA, was also studied and compared with other results.

The features of conjugated diene formation curves for LDL under irradiation were typical of lipid peroxidation curves and were composed of a lag phase (low oxidation phase), followed by a rapid increase in the rate of oxidation (**Fig. 3**). In DHEA-enriched LDL, conjugated diene formation was completely inhibited up to a radiation dose of 50 and 100 Gy ($P < 0.05$) for added DHEA concentrations of 0.1 and 1 μM, respectively. Lipid peroxidation rates, measured from the linear part of the curves, were also significantly reduced in the presence of DHEA ($P < 0.05$). Ethanol alone in LDL, used as a control, also reduced LDL oxidation. Ethanol is well known as a free radical scavenger.

At high radiation doses, conjugated diene formation in LDL without DHEA or ethanol reachs a plateau. On the other hand, in the DHEA-enriched LDL, the formation of conjugated dienes increased gradually up to 200 Gy and then increases quickly, at a radiation dose of 300 Gy, to reach a maximum without reaching a plateau.

Hydroperoxide formation was also reduced and the lag phase was prolonged in the presence of DHEA at a concentration of 0.1 or 1 μM (**Fig. 4**).

To further assess the oxidation state of LDL with or without DHEA, we measured TBARS formation as a function of radiation dose (**Fig. 5**). In the case of LDL irradiated alone, TBARS formation does not present a lag phase. When ethanol was added to LDL, TBARS formation was reduced. However, when LDL were enriched with DHEA, TBARS formation was completely inhibited up to radiation doses of 50 and 80 Gy for DHEA concentrations of 0.1 and 1 μM, respectively.

At a high radiation dose, TBARS formation reached a plateau in LDL enriched with DHEA. This plateau value was less than 50% of that of LDL irradiated alone.

To try to gain more insight into the effect of DHEA on LDL oxidation, we measured its action on the level of vitamin E (α-tocopherol), which is a major endogenous antioxidant in LDL. The oxidation of LDL in solution alone resulted in a significant loss of α-tocopherol (**Fig. 6**). The yield of disappearance of vitamin E [G(−vit. E)], given by the initial slope of the curve in Fig. 4, was 0.147×10^{-7} mol J^{-1}. Oxidation of DHEA-enriched LDL resulted in a significant reduction ($P < 0.01$) in the α-tocopherol depletion, G(−vit. E) = 0.128 and 0.108×10^{-7} mol J^{-1}, when DHEA was added to plasma, before LDL oxidation, at concentrations of 0.1 and 1 μM, respectively. At high radiation doses, up to 100 Gy, α-tocopherol disappeared completely in LDL. However, in DHEA-enriched LDL vitamin E still remained, even at high radiation doses. The concentration of vitamin E remaining in LDL was DHEA concentration dependent. **Table 2** summarizes the princi-

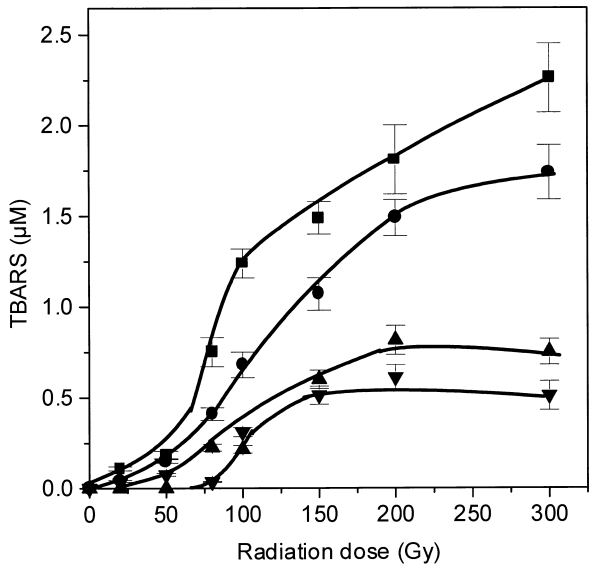

Fig. 5. TBARS formation in LDL from elderly subjects as a function of radiation dose. LDL were enriched with DHEA by direct supplementation of DHEA to plasma before LDL separation. Free radicals were generated by γ radiolysis of water (34 mM ethanol). The concentration of LDL was 100 μg/ml in aerated aqueous solutions containing 10^{-2} M sodium phosphate buffer at pH 7. TBARS concentrations were calculated as malondialdehyde (MDA) equivalents, using the MDA standard curve. MDA was generated by the hydrolysis of 1,1,3,3-tetraethoxypropane. DHEA (0.1 and 1 μM) had no effect on the standard curve. LDL alone (solid squares); LDL plus 34 mM ethanol (solid circles); LDL enriched with 0.1 μM DHEA (solid triangles); LDL enriched with 1 μM DHEA (solid inverted triangles). Results are presented as pooled data from 10 independent experiments performed in duplicate (means ± SD).

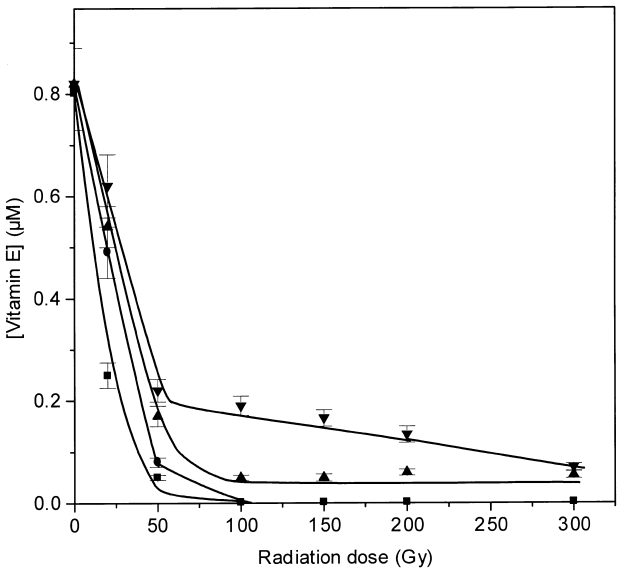

Fig. 6. Vitamin E disappearance from LDL of elderly subjects as a function of radiation dose. LDL were enriched with DHEA by direct supplementation of DHEA to plasma before LDL separation. Free radicals were generated by γ radiolysis of water (34 mM ethanol). The concentration of LDL was 100 μg/ml in aerated aqueous solutions containing 10^{-2} M sodium phosphate buffer at pH 7. Vitamin E was assayed as α-tocopherol, before and after irradiation, by reversed-phase HPLC, with UV detection at 292 nm and electrochemical detection. LDL alone (solid squares); LDL plus 34 mM ethanol (solid circles); LDL enriched with 0.1 μM DHEA (solid triangles); LDL enriched with 1 μM DHEA (solid inverted triangles). Results are presented as pooled data from 10 independent experiments performed in duplicate (means ± SD).

pal results after the oxidation of native or DHEA-enriched LDL.

The contents of DHEA in the LDL were also monitored according to the amount of irradiation. Three concentrations of DHEA were studied in this case (0.1, 1, and 10 μM). **Figure 7** shows the disappearance of the DHEA contained in the LDL according to the amount of irradiation. The values are expressed as a percentage of DHEA compared with its concentration in the LDL in absence of irradiation.

DISCUSSION

In the present study, we show that vitamin E is unable to restore the decreased resistance to oxidation of LDL from elderly subjects to the level of LDL obtained from young subjects. It was then supposed that other factors should be responsible for the increased susceptibility of LDL from elderly subjects to oxidation compared with LDL of young subjects. Here, we demonstrate for the first time that the content of DHEA decreases progressively in LDL with age,

TABLE 2. Effect of DHEA enrichment of an elderly subject LDL oxidized by γ radiolysis of water, expressed as yields of conjugated diene, TBARS formation, and vitamin E disappearance

	Vitamin E		Conjugated Dienes (CD)		TBARS	
	G (−vit. E)	[Vit. E] ≤0.1 μM	Lag Phase	G(CD)	Lag Phase	G (TBARS)
LDL alone[a]	0.147	50 Gy	—	1.709	—	0.069
LDL + D (0.1 μM)	0.128[b]	100 Gy	50 Gy[c]	0.776[d]	50 Gy	0.050
LDL + D (1 μM)	0.108[b]	300 Gy	100 Gy	0.776[d]	80 Gy	0.039

Abbreviations: D, dehydroepiandrosterone; G, initial radiation yields, determined as the initial slopes of the curves and the slopes of the linear part of the curves and expressed as the decrease in α-tocopherol and the amount of conjugated dienes or TBARS formed per unit of energy (joule) absorbed (mol $J^{-1} \times 10^{-7}$), respectively.

[a] Ethanol, 34 mM.

[b] $P < 0.01$.

[c] $P < 0.02$.

[d] $P < 0.05$.

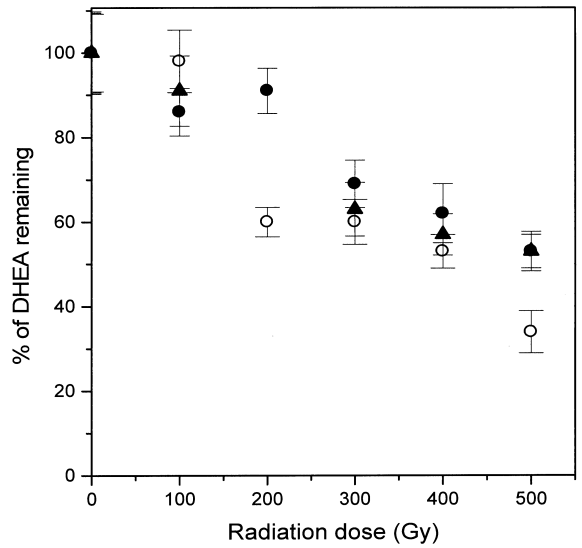

Fig. 7. Effect of $^{\cdot}OH$, $O_2^{-\cdot}$, and $RO_2^{\cdot}$ free radicals produced by γ radiolysis on incorporated DHEA disappearance from LDL. DHEA, at different concentrations (0.1, 1, and 10 μM), was incubated with plasma from elderly subjects before LDL isolation. DHEA was then analyzed in native and oxidized LDL by radioimmunoassay (RIA). 0.1 μM DHEA (open circles); 1 μM DHEA (solid circles); 10 μM DHEA (solid triangles). Values are expressed as means ± SD.

as has already been well demonstrated in sera (60). Moreover, the addition of DHEA ex vivo in the plasma of elderly subjects can restore the DHEA content in LDL and contribute to the protection of the LDL of elderly subjects from oxidation.

Lipid peroxidation is at the origin of the oxidative modifications of LDL (ox-LDL), which have been demonstrated to be an important risk factor for the development of atherosclerosis (61, 62). It is well known that atherosclerosis leads to devastating clinical consequences that increase with age. Many factors may explain this phenomenon, including the increased susceptibility of LDL to oxidation with age (14). This susceptibility of LDL to oxidation may be influenced by their composition, structure, size, and content of antioxidants, the most important being vitamin E (63). In fact, this increased susceptibility with aging can be partly explained by the modifications at the level of fatty acid composition and a diminution in antioxidant, that is, vitamin E content (14).

These observations lead to the first series of our experiments, carried out in the present work, namely the addition of 100 μM vitamin E to the LDL of young and elderly subjects before oxidation by γ radiolysis. It is of note that this concentration represents about three times the mean vitamin E plasmatic concentration. The concentration of DHEA used (0.1 μM) is also three times higher than its concentration in plasma. Our results showed that vitamin E decreased significantly ($P < 0.01$) the susceptibility of LDL to oxidation from elderly as it also did for LDL of young subjects, but this remained even higher than that of LDL from young subjects without vitamin E supplementation. Indeed, vitamin E is responsible for only 30% of the susceptibility of LDL to oxidation (64). Other factors should play a role in the increased susceptibility to oxidation of LDL from elderly subjects compared with young subjects, for instance, the existence of other antioxidants in LDL.

Epidemiological and experimental studies have shown that the susceptibility of LDL to oxidation and the associated pathological effects decrease with the use of antioxidants (65–68). In fact, several studies have reported an inverse association between DHEA and DHEAS plasma levels and the clinical manifestations of coronary and cerebral atherosclerosis (69) as well as mortality (31), while others have failed to demonstrate such association (70). Moreover, studies carried out in experimental animals pointed also to the antiatherogenic effects of DHEA administration (32). Furthermore, studies, including our own (42), suggest that DHEA may act as an antioxidant in vitro. Taken together, the results from human and animal studies suggest a strong antiatherogenic activity of DHEA/DHEAS (71, 72). The hypothesis that the antiatherogenic activity of DHEA/DHEAS is mediated by improvement of lipid metabolism has been proposed, but the mechanism of its antiatherogenic activity is not yet elucidated (73). Thus, the question arises whether the in vivo administration of DHEA can decrease the susceptibility of LDL to oxidation associated with aging. The present study examines the effect of ex vivo addition of DHEA to the sera of elderly subjects before LDL oxidation.

At 21 years of age, serum DHEA and DHEAS levels are about 27 nmol/liter and 10 μmol/liter, respectively (74, 75). They steadily and markedly decrease to reach 10–20% of their peak values by the age of 70 years (76, 77). In pursuing our investigations to elucidate the determinants of the increased susceptibility of LDL to oxidation with age, we measured the DHEA content of LDL at various ages. We have shown that DHEA is an integral constituent of the LDL and that its content decreased with aging to an almost nonmeasurable level (Fig. 2) after 65 years of age and more.

We have already demonstrated that in vitro addition of DHEA was able to decrease the susceptibility to oxidation of LDL from young subjects in a concentration-dependent manner. Our present results demonstrate that the ex vivo addition of DHEA to sera of elderly subjects is able to decrease, in a concentration-dependent manner, the susceptibility of LDL subjected to oxidation by γ radiolysis. The lag phase for conjugated diene formation, which measures the resistance to oxidation of LDL, increased 50 times at a DHEA concentration of 0.1 μM and 100 times with a DHEA concentration of 1 μM. The increase in the lag phase was similar for TBARS formation. The lag phase with 100 μM vitamin E was comparable to that obtained with 0.1 μM DHEA. Nevertheless, the oxidizability of LDL was significantly less with any concentration of DHEA compared with vitamin E. A plateau in the formation of conjugated dienes was reached after 160 Gy with vitamin E addition, while the plateau was reached after 300 Gy in the case of DHEA addition. This suggests a higher efficacy for the LDL to resist oxidation in the presence of DHEA than in the presence of vitamin E. This decreased oxidiz-

ability was also confirmed by the measurement of TBARS in the presence of DHEA. The G values also confirm the efficacy of DHEA in decreasing, in a concentration-dependent manner, the susceptibility of LDL from elderly subjects to oxidation. It was proposed that DHEA at higher concentrations may have a prooxidant effect (40). However, in the range of DHEA concentration studied in this work, this effect was not detected.

These results are in accordance with those obtained in vitro with the LDL of young subjects, where DHEA decreased their susceptibility to oxidation in a concentration-dependent manner (34), as well as with those obtained with the LDL of rats (44). Thus, these results could explain why in the rabbit model of accelerated atherogenesis the administration of DHEA could significantly retard the atherosclerosis in the arteries (32). No direct human data exist on the effects of the administration of DHEA in relation to atherosclerosis or vascular diseases. The only parameters measured during replacement studies were lipoproteins in the case of women and showed a decrease in HDL and apolipoprotein A levels (78). Nevertheless, epidemiological data exist (the Rancho Bernardo cohort) to link the decrease of DHEA/DHEAS with aging to increased incidence of vascular diseases (32). However, some other studies are not so conclusive on the supposed association between DHEAS and cardiovascular protection (79).

Furthermore, studies have already reported that replacement of estrogen, another hormone that disappears with age, in postmenopausal women induced an ex vivo resistance of LDL to oxidation (80). This seemed to be due to the incorporation of estradiol in the LDL (80).

To gain further information on the possible mechanism by which DHEA decreases the susceptibility of LDL from elderly subjects to oxidation after their incorporation in LDL we measured vitamin E and DHEA content. Our results show that the vitamin E disappearance (G values) was significantly decreased after the incorporation of DHEA in LDL. DHEA, even at 300 Gy, was able to prevent the complete disappearance of the vitamin E content in LDL. These results are in accordance with other studies including ours and suggest that DHEA/DHEAS prevents vitamin E loss (42, 44, 81). Moreover, we show that the incorporated DHEA also disappears during LDL oxidation, contributing to the direct radical-scavenging effect of the protection of the LDL from oxidation, as we have already demonstrated (42). The antioxidant mechanism of DHEA seems to be complex and acts on other antioxidants, such as vitamin E, and is itself a scavenger.

In conclusion, our results provide evidence that DHEA is an integral part of LDL and HDL and disappears to almost nondetectable levels with age but can be incorporated ex vivo into both LDL and HDL. The DHEA incorporated into LDL from elderly subjects increased their resistance to oxidation in a concentration-dependent manner. The increased resistance provided by DHEA was higher than that provided by vitamin E. Thus, DHEA exerts an antioxidative effect on LDL, which could have antiatherogenic consequences. Careful clinical trials of DHEA replacement should determine whether this ex vivo effect could be translated into any measurable antiatherogenic (cardioprotective) action.

The authors thank Lise Trottier for excellent technical assistance with the statistical analysis. This work was supported by a grant from the Fonds International de Cooperation Universitaire–FICU (AUPLF-URF, Agence Francophone pour l'Enseignement Supérieur et la Recherche) and by NATO (North Atlantic Treaty Organization) (ref. CRG 970361) as well as by a grant-in-aid from the Centre de Recherche en Gérontologie et Gériatrie de Sherbrooke. This support is gratefully acknowledged. T.F. and A.K. are Senior and Junior-1 fellows, respectively, of the FRSQ.

Manuscript received 20 January 2000 and in revised form 11 May 2000.

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