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Role of β_2-Glycoprotein I, LDL$^-$, and Antioxidant Levels in Hypercholesterolemic Elderly Subjects

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ABSTRACT

The levels of electronegative low-density lipoprotein (LDL$^-$), LDL cholesterol oxidability, and plasma levels of molecular antioxidants and of β_2-glycoprotein I (β_2 GPI) were studied in a group of 10 hypercholesterolemic (HC) and 10 normocholesterolemic (NC) elderly subjects. HC subjects showed significantly higher levels of cholesterol, LDL cholesterol, LDL$^-$, and β_2GPI than NC, whereas high-density lipoprotein cholesterol and α-tocopherol levels were lower in HC as compared with NC subjects. Correlations among LDL$^-$ levels, LDL oxidation lag time, β_2GPI, and antioxidant plasma levels were studied in 100 HC elderly subjects. Lag time for *in vitro* LDL oxidation positively correlated with ubiquinol-10 levels ($p = 0.008$), but not with other antioxidants studied or β_2GPI. LDL$^-$ and α-tocopherol levels showed an inverse and significant correlation ($p = 0.018$). β_2GPI and LDL cholesterol levels were correlated ($p = 0.001$), whereas no significance was found between LDL$^-$ and β_2GPI levels ($p = 0.057$). The physiological significance of α-tocopherol and ubiquinol-10 levels on LDL$^-$ levels, and the presence of high levels of β_2-GPI, are discussed in terms of protective mechanisms operating during the overall atherosclerosis process. *Antioxid. Redox Signal.* 6, 237–244.

INTRODUCTION

CARDIOVASCULAR DISEASES (CVD) are the main cause of adult mortality and have the greatest impact on the elderly population (60). Although CVD represent a heterogeneous group of clinical entities, they share a common mechanism and manifestation, namely, atherosclerosis (37). Atherogenesis can be regarded as the outcome of multiple interactions among inflammatory stimuli, components of the hemodynamic environment, genetic factors, and the resulting reparative responses of the arterial wall that occur in a dyslipoproteinemic environment (50).

Hypercholesterolemia manifests in a series of clinical situations during which vascular modifications arise from endothelial injury and contribute to the progression of atherosclerosis. Lesion initiation and progression are related to excess lipoprotein influx, as well as oxidative damage to low-density lipoprotein (LDL) in the vascular intima (48). Current knowledge on lipoprotein oxidation (16, 54) has offered a plausible explanation for atherosclerosis, prompting researchers to study the role of oxidants and antioxidants in the development of CVD (17).

The hazardous process of LDL oxidation occurs normally within the subendothelial space after interaction with metal

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complexes, reactive oxygen and nitrogen species, some reactive oxidized thiols, hypochlorous acid, and other biological oxidants (25, 26, 41, 56). In the extracellular fluid of the subendothelial space, oxidized LDL (ox-LDL) is removed by macrophages. Otherwise it can damage endothelial cells, increase platelet aggregation and leukocyte adhesion, and promote inflammation (17).

Mildly oxidized LDL also exists within the subendothelial space and is likely related to LDL$^-$, which is also referred to as electronegative or minimally modified LDL (49). Thus, LDL accumulation at specific sites in the arterial wall provides the substrates within an environment that facilitates the formation of LDL$^-$ (49). LDL$^-$ is found in peripheral blood (27, 58), is characterized by minimal to moderate lipid oxidation (27), and shares some of the deleterious biological effects of ox-LDL (17, 31). However, LDL$^-$ is recognized by receptor BE as nonoxidized LDL, whereas ox-LDL is actively assimilated by macrophages through the scavenger receptor pathway (11, 40). Both LDL$^-$ and ox-LDL induce expression and release of chemotatic proteins, adhesion molecules, and growth factors in leukocytes, smooth muscle cells, and endothelial cells (17).

LDL oxidation *in vivo* can be prevented by a number of mechanisms, including compartmentalization of lipid particle metabolism, particle lipid-soluble antioxidants, and plasma antioxidant recycling systems (15). Inside the vascular compartment, LDL is protected from oxidation mainly by its molecular antioxidants (15, 16, 46), by endothelial cell-derived nitric oxide ($NO^{\cdot}$) (45), and by soluble plasma antioxidants and other proteins. LDL resistance to oxidation is greatly determined by the LDL-associated antioxidants, α-tocopherol being the major lipid-soluble antioxidant in LDL particles. On a molar basis, each LDL particle contains five to nine α-tocopherol molecules (15, 16). Other antioxidants (lipid-soluble) are present in the particle, including γ-tocopherol, ubiquinol-10, β-carotene, lycopene, cryptoxanthine, and α-carotene (16). In addition, LDL polyunsaturated fatty acids, as well as LDL lipid-soluble antioxidants, vary considerably among individuals, and are generally dependent on dietary fat intake and rate of fat absorption (13). A number of epidemiological studies in recent years have described the prevention of CVD by lipid-soluble antioxidants; however, the beneficial effects of antioxidants have yielded conflicting results (62).

β_2-Glycoprotein I (β_2GPI) was recently proposed as an antioxidant-like molecule, inhibiting LDL uptake by macrophages through the scavenger receptor pathway (32). β_2GPI, a circulating protein from the complement control family of proteins, is implicated as a versatile antiatherogenic apolipoprotein, but its physiological function remains undetermined (7, 19, 52, 53, 61). It is composed of four typical short consensus repeats and a positively charged fifth domain, through which β_2GPI binds to the negatively charged surfaces of activated platelets and to oxidized lipoproteins (47). It activates endothelial lipoprotein lipase, and increases *in vivo* clearance of chilomicrons and very low-density lipoproteins (VLDL) (36). The protective effect of lipoprotein lipase activation on LDL-mediated atherogenic responses was recently described (63). There are well documented reports on β_2GPI antithrombotic properties; it directly inhibits platelet activation and contact phase coagulation (7, 34). This apolipoprotein has been found in stabilized plaques and was proposed to modulate lymphocyte response in late lesions (20, 53). On the other hand, β_2GPI is a known coantigen for the thrombogenic antiphospholipid pathological antibodies found in some infectious and autoimmune diseases (6, 21). Autoimmune anti-β_2GPI antibodies are frequently associated with atherogenesis (1). It has been speculated that these antibodies modulate metabolic uptake of oxidized lipid products from damaged membranes or lipoproteins (28, 29).

The aim of the current article was to evaluate the levels of LDL$^-$, LDL cholesterol oxidability, plasma levels of molecular antioxidants and of β_2GPI, as well as to study the possible correlation among these parameters, in hypercholesterolemic (HC) elderly subjects. The goal is to understand better the protective role of antioxidants and β_2GPI in atherosclerosis.

PATIENT SELECTION AND METHODS

Subject selection and sample collection

All subjects were selected among the participants of a cohort of elderly residents that have been followed since 1991 at the Center for the Study of Aging of the University Federal São Paulo (UNIFESP), as part of the EPIDOSO project (44). At the beginning of this specific study (1999), corresponding to the third cycle of the EPIDOSO study, 100 subjects (mean age 78 ± 5 years old, 65% female with LDL cholesterol ≥ 130 mg/dl) were invited to participate in EPIDÓXI Project (UNIFESP Ethics Committee Report no. 718/98). A group of 10 subjects fulfilling all the above criteria, but presenting levels of LDL cholesterol < 130 mg/dl, were also studied in order to calibrate the methods for the elderly population. The following conditions excluded subjects from this study: secondary dyslipidemia, triglyceride plasma levels > 400 mg/dl, fever, unstable vascular disease (heart attack, stroke), disability (dementia, which could make it impossible to follow the recommendations), and intake of vitamin supplements and/or statins. The participants were scheduled for blood collection after 12 h of fasting, and the following measurements were performed: lipid profile [triglycerides, total cholesterol, and high-density lipoprotein (HDL), LDL, and VLDL cholesterol; samples collected without anticoagulant], vitamin plasma levels (blood collection on heparin), LDL$^-$, and LDL oxidability (blood collection on EDTA).

Determination of lipid-soluble antioxidants

Lipid extracts from plasma samples were prepared with methanol/hexane using the procedure described by Burton and Ingold (9). The pellet obtained after final solvent evaporation was dissolved in 0.4 ml of methanol/ethanol (1:1, vol/vol) for injection into an HPLC (30). Aliquots of 20 μl were injected onto a 3.9 × 150 mm C8 Nova-Pak column, under isocratic mobile phase delivery [20 m*M* $LiClO_4$ in methanol/water (98:2, vol/vol, 0.7 ml/min)]. A BAS electrochemical detector was used, with an oxidation potential of 0.6 V. The observed retention times were, respectively (in min): γ-tocopherol, 3.33; α-tocopherol, 3.56; lycopene, 5.54; β-carotene, 7.34; and

ubiquinol-10, 10.5. Antioxidant concentrations were calculated by comparing the sample area with the corresponding standard area, and expressed in molar concentrations. Plasma concentrations of lipid-soluble antioxidants were further corrected for LDL cholesterol levels.

Measurement of plasma vitamin C

Vitamin C from heparinized plasma samples was extracted with 10% HPO_3 and analyzed by HPLC (57). Aliquots of 20 µl were injected onto a 3.9 × 300 mm C18 µBondapak column coupled to an isocratic delivery system (1.0 ml/min of 0.8% HPO_3). Detection was accomplished using a Waters 484 UV detector set at 240 ηm. The retention time for ascorbic acid was 3.18 min. Concentrations of ascorbic acid were calculated by comparing the sample area with the corresponding standard area, and the results expressed in micromolar.

LDL separation from plasma

Blood collected into EDTA (1 mg/dl) was centrifuged at 700 *g* at 4°C for 15 min. Plasma was removed and transferred to an ultracentrifuge tube and the total volume measured. Protease inhibitors were added to all plasma samples (phenylmethylsulfonyl fluoride, 10 µl/ml of plasma; benzamidine, 4 µl/ml of plasma; and aprotinine, 2 µl/ml of plasma). LDL separation was based on a method using KBr gradient ultracentrifugation (24). LDL fractions were collected and dialyzed overnight against 0.9% NaCl containing Chelex, at 4°C. At the end of dialysis, protein content of the samples and LDL oxidability were measured. Remaining LDL was used for LDL⁻ separation by HPLC.

LDL oxidability evaluation

Oxidation was followed by conjugated diene formation at 234 nm. Lag-phase measurements and first-order oxidation rates were estimated according to Esterbauer *et al.* (14, 16). Lag phase (minutes) corresponds to the time interval between copper addition (zero time) and the increasing absorbance (propagation phase). Oxidation rates were calculated from the curve tangent intersection with the *x*-axis and expressed as Δ absorbance/min.

HPLC separation of LDL⁻

Samples of 300–500 µg of protein from isolated LDL fractions were injected onto an HPLC system (1 ml) to separate LDL from LDL⁻. Separation and elution were performed at 1.0 ml/min, using a 50 × 7.8 mm ion-exchange column (Bio-Rad MA7Q) and a NaCl gradient from 0 to 0.3 *M* in Tris-HCl (0.01 *M*) (27). Eluted protein was followed at 280 nm using an UV/Vis Waters 2487 detector. Peak integration was done using the Data Station Unicam GC system.

Serum levels of total cholesterol and lipoprotein fractions

Measurement of serum cholesterol and lipoprotein fractions was performed using commercial enzymatic reagents and a Technicon autoanalyzer model R4100.

Protein measurement

The amount of protein in isolated LDL fractions was measured according to Lowry *et al.* (33).

β_2GPI measurement in plasma

β_2GPI concentrations in plasma of elderly hyperlipidemic patients were measured by a competition ELISA method, developed in our lab. Nunc plates were coated with 0.1 µg of purified β_2GPI/well (43). Coated plates were washed three times with phosphate-buffered saline and blocked with 5% milk solution for 2 h. Assays (in triplicate) were performed as follows: 50 µl of ICN Pharmaceuticals anti-apolipoprotein H monoclonal antibody 5F7 (1:10,000) and 50 µl of plasma samples (1:200) were added to each well, incubated for 1 h at 37°C, washed three times with phosphate-buffered saline, and incubated with a Pharmingen Becton–Dickinson Co. anti-mouse IgG peroxidase (1:5,000; 100 µl/well). Reactions were revealed with tetramethylbenzidine (Dade Behring TMB Kit) and measured at 450 nm in a microplate reader Bio-Rad 3550. A standard curve was created using increasing concentrations of purified β_2GPI (0.01–1.5 µg/well).

Statistical analysis of data

Significant differences between control and HC patients were determined using Student's *t* test. Power of the sample $(1 - \beta)$ calculations were made by the UNIANOVA procedure, as provided by the SPSS program. When needed, log transformation and Box–Cox transformations were used to stabilize the variance. Correlation between parameters was evaluated by the Pearson χ^2 test. Nonnormal data were analyzed by the Spearman test. Differences were considered significant for $p < 0.05$.

RESULTS

The cohort was initially studied on the basis of their plasma lipid profile, considering previous data on age, gender, and ethnic traits (44). To avoid systematic influence of preventable bias, the elderly population parameters were studied first within a group of 10 normocholesterolemic (NC)–HC subject pairs, matched for age, gender, ethnic origin, blood pressure, and glycemia. The correlations among the LDL, antioxidant, and β_2GPI plasma levels, LDL⁻, and LDL oxidability were studied in the whole group of 100 HC elderly individuals.

Elderly HC patients presented significantly higher levels of cholesterol, LDL cholesterol, LDL⁻, and β_2GPI than NC, whereas HDL cholesterol was lower in HC as compared with NC (Table 1). Both groups presented equivalent LDL lag times and oxidation rates (Table 1). LDL oxidation lag phase is reported to depend on antioxidant content, whereas oxidation rates are related to the unsaturated fatty acid composition of the LDL particles (16). Plasma levels of antioxidant vitamins were equivalent, except for α-tocopherol levels, which were significantly lower in HC (Table 2) based on LDL cholesterol content.

Correlations among LDL⁻ levels, LDL oxidation lag time, β_2GPI, and antioxidant plasma levels were determined within

TABLE 1. LIPID PROFILE, LDL OXIDATION, AND β_2GPI LEVELS IN ELDERLY SUBJECTS ($N = 10$)*

	NC	*HC*	*p*	$(1 - \beta)^{\dagger}$
Total cholesterol (mg/dl)	198 ± 13	244 ± 25	<0.001	0.998
Triglycerides (mg/dl)	95 ± 31	147 ± 79	0.070	0.445
VLDL cholesterol (mg/dl)	19 ± 6	28 ± 16	0.073	0.434
HDL cholesterol (mg/dl)	59 ± 10	38 ± 5	<0.001	1.000‡
LDL cholesterol (mg/dl)	120 ± 15	179 ± 23	<0.001	1.000
LDL⁻ (mg/dl)	0.8 ± 0.3	9 ± 6	0.002	1.000§
Lag phase (min)	72 ± 11	69 ± 27	0.724	0.063
Oxidation rate (ΔAbs/min)	0.022 ± 0.007	0.020 ± 0.012	0.702	0.145‡
β_2GPI (μg/ml)	243 ± 116	403 ± 196	0.040	0.554

*Data are shown as means ± SD.

†Power of the sample.

‡UNIANOVA was calculated after variance stabilization by log transformation, because of unequal variance between the compared groups.

§UNIANOVA was calculated after variance stabilization by Box–Cox transformation with $\lambda = 1/4$, because of unequal variance between the compared groups.

the whole HC group. Results obtained for this population are shown in Tables 3 (descriptive data) and 4 (correlation data). Lag time for *in vitro* LDL oxidation positively correlated with ubiquinol-10 levels ($p = 0.008$), but not with other antioxidants studied or β_2GPI (Table 4). LDL⁻ and α-tocopherol levels showed an inverse and significant correlation (Table 4). No significance was obtained for the relationship between LDL⁻ and β_2GPI levels in the HC group ($p = 0.057$).

DISCUSSION

Evidence is accumulating that LDL oxidation impacts individual inflammatory activity, and that this determines atherosclerosis initiation and progression (38, 39, 59). Moreover, Sánchez-Quesada *et al.* (46) showed in patients with heterozygous familial hypercholesterolemia that *in vivo* oxidative modification of LDL particle is prevented by antioxidants. LDL⁻ levels found in that study were almost fourfold increased as compared with those of control subjects. Notably, the use of simvastatin produced a decrease in LDL⁻, as well as in total cholesterol and LDL cholesterol during treatment.

A preventive role of lipid-soluble antioxidants in *in vivo* LDL oxidation is also shown in this study. HC elderly patients showed an almost 10-fold increase in LDL⁻ concentrations as compared with their NC pairs, with a similar LDL particle antioxidant loading between the two groups, except for lower α-tocopherol concentration (Tables 1 and 2). Accordingly, α-tocopherol was the only antioxidant that presented a significantly inverse correlation with LDL⁻ levels among the HC elderly (Table 4).

The two studied groups do not differ with respect to the *in vitro* LDL oxidation lag phase or rate of oxidation (Table 1). Despite literature evidence for a strong dependence on Cu^{2+} concentrations for the induction and rate of LDL oxidation, and on the content of lipid-soluble antioxidants (18), no correlation among lag phase and lipid-soluble antioxidants was found in this study, with the exception of ubiquinol-10 (Table 4). These differences may be partially explained by the fact that LDL resistance to oxidation depends not only on its α-tocopherol content, but also on the composition of other antioxidants, polyunsaturated fatty acids, preformed peroxides, cholesterol/protein ratio, and distribution of LDL subfractions (17). In this respect, the content of LDL⁻ in the LDL fraction has a strong effect on the oxidizability of the total LDL pool without having a significant effect on the total α-tocopherol content of the pool (51).

It is noteworthy that ubiquinol-10 is a minor component of the particle antioxidant defenses, whereas tocopherols are the main antioxidants within LDL particles (16, 18). However,

TABLE 2. PLASMA ANTIOXIDANTS IN ELDERLY PATIENTS ($N = 10$)*

	NC	*HC*	*p*	$(1 - \beta)^{\dagger}$
α-Tocopherol (nmol/mg of LDL cholesterol)	14 ± 4	9 ± 3	0.005	0.869
γ-Tocopherol (nmol/mg of LDL cholesterol)	2.6 ± 1.1	2.0 ± 0.8	0.181	0.261
Ascorbic acid (μ*M*)	56 ± 16	60 ± 21	0.648	0.072
Lycopene (nmol/mg of LDL cholesterol)	0.4 ± 0.3	0.3 ± 0.1	0.238	0.211
Ubiquinol-10 (nmol/mg of LDL cholesterol)	0.16 ± 0.12	0.10 ± 0.05	0.095	0.386
β-Carotene (nmol/mg of LDL cholesterol)	0.6 ± 0.6	0.4 ± 0.3	0.395	0.131‡

*Data are shown as means ± SD.

†Power of the sample

‡UNIANOVA was calculated after variance stabilization by log transformation, because of unequal variance between the compared groups.

TABLE 3. LDL OXIDATION, PLASMA ANTIOXIDANTS, AND β_2GPI LEVELS IN HC ELDERLY SUBJECTS (N = 100)*

LDL cholesterol (mg/dl)	182 ± 31
LDL⁻ (mg/dl)	8 ± 6
Lag phase (min)	68 ± 23
Oxidation rate (ΔAbs/min)	0.03 ± 0.01
Ascorbic acid (μ*M*)	57 ± 23
α-Tocopherol (μ*M*)	20 ± 6
α-Tocopherol (nmol/mg of LDL cholesterol)	11.4 ± 4.1
γ-Tocopherol (μ*M*)	3.2 ± 1.2
γ-Tocopherol (nmol/mg of LDL cholesterol)	1.83 ± 0.80
Lycopene (μ*M*)	0.66 ± 0.35
Lycopene (nmol/mg of LDL cholesterol)	0.37 ± 0.21
Ubiquinol-10 (μ*M*)	0.17 ± 0.12
Ubiquinol-10 (nmol/mg of LDL cholesterol)	0.092 ± 0.067
β-Carotene (μ*M*)	0.75 ± 0.47
β-Carotene (nmol/mg of LDL cholesterol)	0.41 ± 0.26
β_2GPI (μg/ml)	408 ± 171

*Data are shown as means ± SD.

ubiquinol-10 consumption occurs very early during *in vitro* LDL oxidation (55). It is important to note that dietary supplementation with ubiquinol-10 increases LDL resistance to oxidation (35). Nevertheless, it remains uncertain whether in this study low concentrations of ubiquinol-10 significantly contributed to the LDL oxidation lag phase. The role of ubiquinol-10 in the extent of LDL oxidizability may have been biased, to some extent, by the very low levels of ubiquinol-10 (Tables 2 and 3) found in this cohort as compared with other reports (8). However, these elderly subjects take a diet that is poor in ubiquinol-10. Main sources are only 175 g of bovine meat and 70 g of fish per week, plus low intake of vegetable oils, for both groups (personal communication). As evidenced by the amount of circulating molecular antioxidants, the levels of LDL⁻ and *in vitro* LDL oxidability described above, it remains clear that these last two parameters do not give strictly equivalent physiopathological information. The extent of *in vivo* LDL oxidation probably correlates better with LDL⁻ plasma concentration than with *in vitro* LDL oxidation lag phase or rate.

TABLE 4. CORRELATION STUDIES IN HC ELDERLY SUBJECTS (N = 100)*

	Pearson coefficient	*Spearman coefficient*	*p*
LDL × β_2GPI	0.339		0.001
LDL⁻ × LDL	0.288		0.004
LDL⁻ × α-tocopherol		−0.295	0.003
LDL⁻ × γ-tocopherol		0.041	0.689
LDL⁻ × β-carotene	0.076		0.457
LDL⁻ × lycopene	−0.135		0.184
LDL⁻ × ubiquinol-10	−0.124		0.221
LDL⁻ × β_2GPI	0.205		0.057
Lag phase × α-tocopherol		−0.103	0.313
Lag phase × γ-tocopherol		0.091	0.373
Lag phase × β-carotene	0.045		0.659
Lag phase × lycopene	0.030		0.767
Lag phase × ubiquinol-10	0.266		0.008
Lag phase × β_2GPI	−0.013		0.905
Oxidation rate × β_2GPI	0.085		0.431

*Concentrations of lipid-soluble antioxidants used for the correlation studies were those corrected by LDL cholesterol.

In addition to the well-known antioxidants, Lin *et al.* (32) proposed that β_2GPI may play an antioxidant role against LDL oxidation. β_2GPI plasma levels are higher among HC subjects than their NC pairs (Table 1). NC elderly subjects showed β_2GPI plasma levels closely comparable to those described by several independent groups (2, 5–7, 61). Moreover, increased levels of the protein found among HC subjects (Tables 1 and 3) are comparable to those found by some authors in autoimmune disease patients (2, 6), although other authors report lower values (5, 32).

According to Lin *et al.* (32), β_2GPI plasma levels directly correlate with the extent of LDL oxidation lag phase. We did not demonstrate this effect, as no correlation was found within the two parameters in the HC subjects studied (Table 4). These mentioned differences are possibly due to the subjects selected for both studies. Patients from Lin's study are symptomatic CVD patients, presenting lower LDL cholesterol levels than the subjects in this study. β_2GPI is implicated in the differential regulation of ox-LDL-associated cholesterol influx and efflux in J774 macrophages (32). Taken together, the findings to date show a consistent relationship for high concentrations of β_2GPI among HC elderly asymptomatic subjects, a significant correlation between β_2GPI and LDL cholesterol, and no correlation between LDL⁻ and β_2GPI plasma levels (Table 4). This suggests that although β_2GPI cannot be excluded as an antioxidant molecule, other functional effects must be considered, and metabolic effects on LDL that are influenced by other factors acting on LDL may strongly affect the relationship between β_2GPI levels and LDL oxidizability. Alternatively, lipid-soluble antioxidants and β_2GPI may act through independent protection mechanisms.

A possible antiinflammatory role for β_2GPI has been suggested by Balasubramanian and Schroit (4) and Gomes *et al.* (22). Indeed, we demonstrated that β_2GPI increases uptake and degradation of liposome particles by nonparenchymal liver cells and inhibits uptake-associated oxygen consumption in these cells. β_2GPI also inhibits macrophage respiratory burst during the phagocytosis of opsonized zymosan (22). Uptake of opsonized particulate material in *ex vivo* perfused mouse liver is even more sensitive to β_2GPI than that in isolated nonparenchymal liver cells (23).

β_2GPI is produced in liver and intestines as an acute-phase inflammatory protein (3). β_2GPI is distributed among lipoprotein fractions (42), being 60% unbound and 40% bound to lipoproteins, mainly VLDL and chilomicrons. β_2GPI distributes to lymph, liquor, and extracellular fluid compartments other than plasma, in which it can affect macrophage functions (43). As cardiolipin and phosphatidylserine residues are found regularly in human lipoproteins, a possible role for β_2GPI in modulating atherogenic properties of these oxidizable lipids cannot be excluded (12). Chonn *et al.* (10) have demonstrated that β_2GPI selectively concentrates in negative particles containing cardiolipin and phosphatidylserine, in human blood. As proposed by Gomes *et al.* (22), binding of β_2GPI to negatively charged particles can promote the silent removal of

these particles by liver macrophages. This may apply also to LDL⁻ as it is negatively charged and has many atherogenic properties. One can hypothesize that if it occurs, binding of β_2GPI to LDL⁻ may facilitate efficient removal of this potentially harmful particle, protecting the vasculature from atherogenesis progression. Thus, increased β_2GPI levels may be a beneficial compensatory response for HC elderly subjects. Despite no correlation between LDL⁻ and β_2GPI levels (Table 4), β_2GPI levels correlate with LDL cholesterol levels. It may be that β_2GPI binding to LDL requires electronegative moieties, but a normal particle structure that permits interaction with specific domains.

In conclusion, data presented in this article show that mild *in vivo* LDL oxidation is related to low levels of α-tocopherol in HC elderly patients. Moreover, an additional mechanism may be operating via the protective and nonatherogenic removal of LDL⁻ by liver cells that may be elicited by species such as β_2GPI that influence LDL metabolism. A removal of LDL⁻ would confer a stronger antioxidant state for the total LDL population by removing the prooxidative particles. Whether the proposed antiatherogenic role of β_2GPI operates in this case remains to be clarified.

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ABBREVIATIONS

CVD, cardiovascular disease(s); β_2GPI, β_2-glycoprotein I; HC, hypercholesterolemic; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDL⁻, *in vivo* minimally modified LDL; NC, normocholesterolemic; ox-LDL, oxidized LDL; VLDL, very low-density lipoprotein.

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