Lipoprotein(a) and lipoprotein profile in healthy centenarians: a reappraisal of vascular risk factors

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ABSTRACT **In this study we assessed whether widely accepted risk factors for atherosclerotic vascular diseases such as lipoprotein(a) [Lp(a)], a cholesterol-rich lipoprotein under strict genetical control, and other lipid parameters change with age. The variations of blood levels and the pathophysiological role of Lp(a) in old people, and particularly in the oldest old, are unknown. Accordingly, we measured Lp(a) levels as well as total, LDL, and HDL cholesterol (CT), and triglycerides (TG) in sera from 75 healthy centenarians, 114 randomly selected subjects under 65 years, 73 randomly selected elderly people, and 30 healthy selected elderly people. The results showed that Lp(a) serum levels did not vary by age group, including centenarians. Remarkably, one-quarter of the centenarians had high Lp(a) serum levels even though they never suffered from atherosclerosis-related diseases. At variance with young and aged people, centenarians with high Lp(a) serum levels also had high plasma concentrations of the proinflammatory cytokine IL-6, suggesting that genetic control of the Lp(a) serum level may attenuate with age and that environmental factors such as chronic subclinical inflammatory processes may play a role. We also showed that most centenarians are paradoxically characterized by low HDL-CT and relatively high TG levels, which together are considered to be strong risk factors for coronary heart disease. On the whole, these data support the hypothesis that a continuous and complex reshaping of lipid metabolism occurs in physiological aging, likely contributing to successful aging.—Baggio, G., Donazzan, S., Monti, D., Mari, D., Martini, S., Gabelli, C., Dalla Vestra, M., Previato, L., Guido, M., Pigozzo, S., Cortella, I., Crepaldi, G., Franceschi, C. Lipoprotein(a) and lipoprotein profile in healthy centenarians: a reappraisal of vascular risk factors.** ***FASEB J.*** **12, 433–437 (1998)**

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HIGH LEVELS OF total serum cholesterol (CT),[3] low density lipoprotein (LDL)-CT, and triglycerides (TG) as well as low levels of high density lipoprotein (HDL)-CT are well-recognized risk factors for cardiovascular disease (1, 2). Recently, lipoprotein(a) [Lp(a)], a genetically controlled cholesterol-rich lipoprotein whose biological role is still unknown (3), has been suggested by numerous studies as an independent risk factor for premature coronary heart disease, stroke, restenosis of vein grafts after coronary bypass surgery, and peripheral artery disease (4–5); Lp(a) is present in atherosclerotic plaques and stimulates smooth muscle cell replication (6). Although individual Lp(a) levels appear to remain remarkably stable, in some pathophysiological states it can behave as an acute-phase protein (7); increased Lp(a) levels have also been found in chronic inflammatory disorders such as rheumatoid arthritis (5). Few and contrasting data have been published about Lp(a) levels and its atherogenic effect in the elderly (8–10).

The aim of this study was to verify whether the previously mentioned, widely accepted and well-studied lipid risk factors for atherosclerosis and atherosclerosis-related diseases change with age. Accordingly, lipoprotein (a) [Lp(a)] levels as well as the lipoprotein profile (total, LDL, and HDL cholesterol) were studied in serum samples from 75 centenarians carefully selected for their health status compared with 217 control subjects of different ages, selected either randomly or according to their good health status.

The main and unexpected findings were: *1*) Lp(a) serum levels did not vary in the different ages, including centenarians; *2*) 25% of the centenerians had high levels of Lp(a); *3*) only centenarians (but not young and aged subjects) with high Lp(a) levels had a significantly high serum level of proinflammatory cytokine IL-6; *4*) in centenarians, total and

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[3] Abbreviations: CT, cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; LP(a), lipoprotein(a); TG, triglycerides; TNF, tumor necrosis factor; IL-6, interleukin 6; ELISA, enzyme-linked immunoassay.

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LDL cholesterol levels were similar to those found in young controls, but significantly lower than those found in randomly selected or healthy elderly, whereas HDL cholesterol was lower and triglycerides higher than in young subjects. As far as we know, these are the first data about lipid parameters in centenarians, and they suggest that the biological significance of vascular risk factors may change with advanced age.

METHODOLOGY

Blood was drawn after 12 h of fasting from four groups of subjects living in Northern Italy; group 1: 75 healthy centenarians (14 males and 61 females) aged 100–106 years ($\bar{X}\pm$SD: 100.9±1.4); group 2: 114 randomly selected subjects under 65 years (39 males and 75 females) aged 8–64 years ($\bar{X}\pm$SD: 35.8±11.8); group 3: 73 randomly selected elderly persons (16 males and 57 females) aged 65–98 years ($\bar{X}\pm$SD: 83.5±7.6); group 4: 30 healthy selected elderly (6 males and 24 females) aged 61–80 years ($\bar{X}\pm$SD: 71.4±5.5). Group 1 was selected according to clinical, biochemical, and hematological criteria and parameters as previously reported (11); part of this group has been studied for other parameters (12). Group 2 included randomly selected volunteers working in the General Hospital, University of Padua, Italy. These subjects were clinically healthy and attending work (students, medical doctors, nurses, technicians). Group 3 included randomly selected, apparently healthy, old volunteers, about half of them living in institutions for the elderly in Padua city. According to the random selection criteria, no biochemical or hematological analysis was performed in groups 2 and 3 to check their healthy status and no questionnaire for medications was used. Group 4 included old volunteers carefully selected according to the SENIEUR Protocol proposed by Ligthart et al. (13). At the onset, a population of 300 subjects living in Carpi (Modena, Italy), participating in a large study of the health status at a population level, was analyzed for clinical health, several biochemical and hematological parameters, and medications. According to the strict exclusion and inclusion criteria, only 30 subjects were eventually enrolled. All subjects in groups 1–4 were Caucasian and lived in Northern Italy.

Lp(a) levels were measured in serum by a noncompetitive sandwich enzyme-linked immunoassay (ELISA) method (14); total cholesterol (15), HDL cholesterol (after polyanion precipitation) (16), and triglycerides (17) were measured manually by standard enzymatic methods, LDL cholesterol was calculated by the Friedewald formula. Tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) were measured in plasma with commercially available ELISA (18).

Statistical analysis

Lp(a) levels were compared between the groups by the nonparametric Wilcoxon ranked sum test because of the well-known skewed distribution of the concentration of this lipoprotein in Caucasians; the Student's *t* test was used to compare lipids, cytokines, and Lp(a) logarithms. The correlation of Lp(a) levels with age was evaluated with simple linear regression analysis.

RESULTS

Table 1 shows that no statistically significant age-related differences in Lp(a) serum concentration were found among the four groups, including centenarians. Indeed, no significant correlations were found between Lp(a) and age when subjects of all four groups were analyzed together. The same result was obtained when centenarians were excluded from the analysis (data not shown). In centenarians, the serum concentration of Lp(a) varied widely among individuals as well as in the three control groups considered here. Moreover, the percent of frequency of subjects with Lp(a) levels below and above 30 mg/dl, the conventional limit of high-risk levels (3), was similar in the four groups. In particular, 25.3% of healthy centenarians had Lp(a) levels higher than 30 mg/dl. To identify the possible reason (or reasons) for this unexpected high level of Lp(a) in healthy centenarians, we measured the plasma level of proinflammatory cytokines such as IL-6 and TNF-α, which are capable of increasing the plasma level of acute-phase proteins. These measurements were performed in the centenarians (group 1), young subjects (group 2), and old people belonging to group 3 (randomly selected) or 4 (selected for healthy status) who had Lp(a) levels higher than 50 mg/dl or lower than 10 mg/dl.

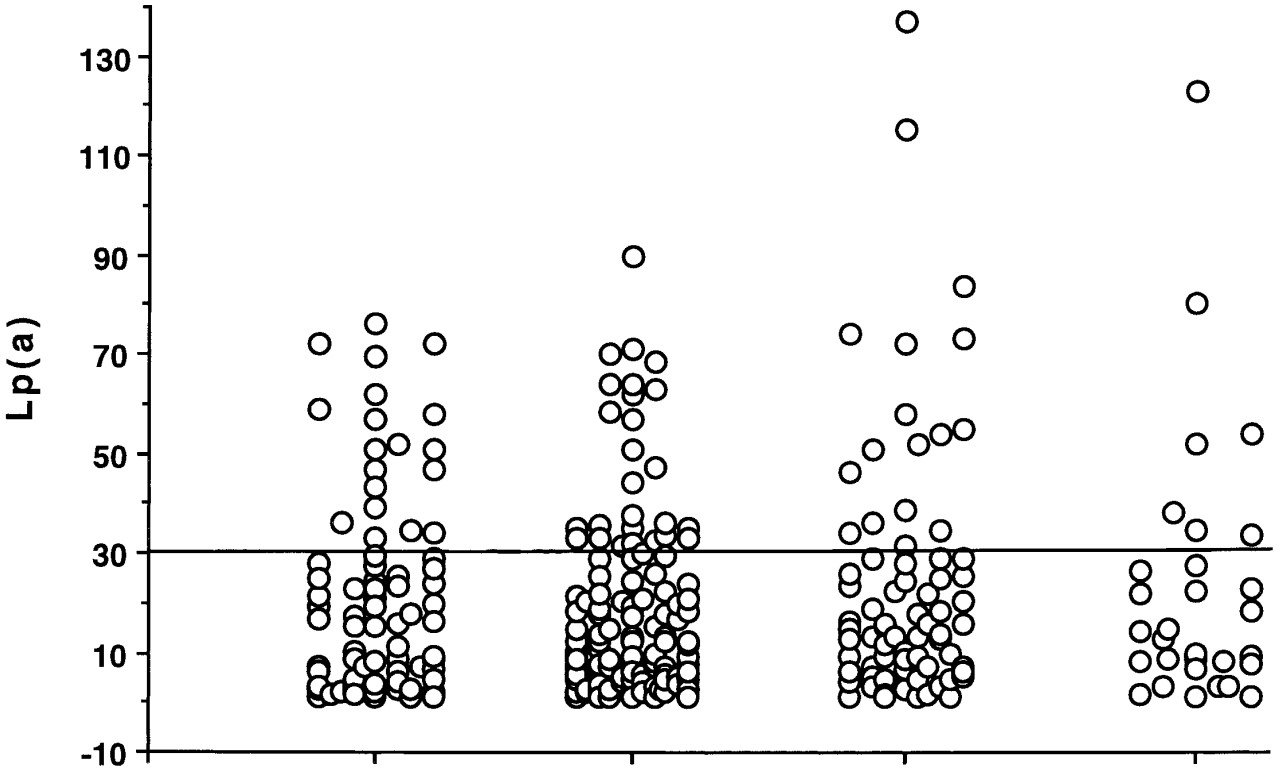
Table 2 shows that significantly higher levels of IL-6, but not of TNF-α, were present only in centenarians with high Lp(a) levels. This increase in IL-6 was not present either in young (group 2) or old subjects (groups 3 and 4) with similar high Lp(a) serum concentrations. A marked increase in the IL-6 serum level was found in randomly selected elderly (group 3) compared with healthy selected old people (group 4). This difference in the serum IL-6 level was also evident when all people belonging to these two groups were analyzed (10.3±0.7 pg/ml vs. 1.8±0.3 pg/ml in 65 subjects of group 3 and 29 subjects of group 4, respectively, $P<0.0001$).

Serum lipid and lipoprotein levels were measured in healthy centenarians as well as in the other three groups considered in this study (**Table 3**). Healthy centenarians showed: *1*) total and LDL cholesterol levels similar to young subjects, but significantly lower than in randomly or healthy selected elderly; *2*) HDL cholesterol values similar to randomly selected elderly, but significantly lower than in young subjects; and *3*) triglyceride levels significantly higher than those found in young people, but similar to elderly subjects.

DISCUSSION

Several studies indicate that altered serum lipid and lipoprotein levels can be considered important risk factors for atherosclerotic vascular diseases (1, 2). In particular, high levels of LDL-CT and low levels of HDL-CT have been described in patients who suffer coronary heart disease (19). More recently, high levels of Lp(a) have been considered an independent

TABLE 1. *Lp(a) levels (mg/dl) in centenarians and controls*



	Centenarians (n=75)	<65 years, randomly selected (n=114)	>65 years, randomly selected (n=73)	>60 years, healthy selected (n=30)
Age range (in years)	100–106	8–64	65–98	61–80
Age mean	100.9 ± 1.4	35.8 ± 11.8	83.5 ± 7.6	71.4 ± 5.5
Lp(a) average	22.4	19.3	23.8	23.0
Lp(a) median	17.2	12.5	15.2	14.2
Lp(a) range	1–76	1–90	1–137	1–123
Log Lp(a) ($\bar{X}\pm$SD)	1.11 ± 0.52	1.06 ± 0.48	1.13 ± 0.51	1.12 ± 0.51
% subjects with				
Lp(a) >30 mg/dl	25.3	22.8	23.3	23.3
Lp(a) <30 mg/dl	74.7	77.2	76.7	76.7

risk factor for atherosclerotic vascular diseases (4–5). The serum level of this lipoprotein is apparently under strict genetic control and shows a poor response to hypolipidemic diet and/or drugs (3, 20). Thus, it could be expected that healthy elderly, and particularly healthy centenarians (people who escaped major age-related diseases, including vascular accidents such as stroke and myocardial infarction), had low levels of Lp(a) as well as a favorable lipoprotein pattern. Moreover, as it is known that all atherosclerotic vascular diseases increase with age (21), it could be expected that serum levels of Lp(a) also change with age. These predictions were only partially fulfilled.

First, the median and mean log of serum Lp(a) levels did not change with age (Table 1). Second, a quarter of healthy centenarians had Lp(a) levels considered to put them at risk for atherosclerosis and related complications. This paradoxical finding apparently challenges the tenet that high levels of Lp(a) are an independent risk factor for the above-mentioned diseases. To fully evaluate these data, we should know whether this portion of centenarians (25%) were characterized for their entire life by such high levels of Lp(a). This question remains unanswered until longitudinal studies are performed. This finding in healthy centenarians who suffered no vascular disease in their long life suggests either that Lp(a) is not a risk factor in the oldest-old or that it is counteracted by unknown protective factors whose identification would be of great interest. Alternatively, it could be speculated that Lp(a) may have a paradoxical protective effect that emerges only later in life in selected people such as healthy centenarians. Such phenomena are not completely unexpected according to one of the most accepted theories of aging: pleiotropic antagonism (22).

TABLE 2. *Interleukin 6 and tumor necrosis factor-α plasma levels in centenarians and controls with high and low Lp(a) levels (x̄ ± SE)*[a]

	Lp(a) (mg/dl)	TNF-α (pg/ml)	IL-6 (pg/ml)	Age (years)
Centenarians				
High Lp(a) (n=8)	65.0 ± 3.09	2.68 ± 0.57	17.66 ± 8.48*	102 ± 0.93
Low Lp(a) (n=12)	4.33 ± 0.81	2.53 ± 0.53	3.11 ± 0.60	101 ± 0.51
Young controls				
High Lp(a) (n=6)	77.8 ± 14.1	1.87 ± 0.35	3.87 ± 0.40	33.0 ± 5.02
Low Lp(a) (n=6)	2.75 ± 0.53	2.40 ± 1.29	3.10 ± 0.28	35.0 ± 2.53
>65 years, randomly selected				
High Lp(a) (n=9)	77.4 ± 10.01	n.d.	9.75 ± 2.01	84.3 ± 3.00
Low Lp(a) (n=26)	4.99 ± 0.58	n.d.	9.75 ± 1.23	84.6 ± 1.56
>65 years, healthy selected				
High Lp(a) (n=4)	77.2 ± 16.53	n.d.	3.43 ± 2.06	71.0 ± 3.03
Low Lp(a) (n=12)	6.13 ± 0.97	n.d.	1.25 ± 0.11	71.8 ± 1.25

[a] $P < 0.05$ vs. centenarians with low Lp(a); n.d. = not determinated.

Another tenet challenged by our data is that Lp(a) is mostly or strictly under genetic control and that environmental influences play a negligible role. On the contrary, the high level of IL-6 only in centenarians with high Lp(a) serum levels, but not in young and aged subjects, suggests that genetic control of the serum level of Lp(a) may attenuate with advanced age where environmental factors, such as chronic subclinical inflammatory process, may be involved (5, 23).

The increased number of activated T cells described in the peripheral blood of centenarians fits this interpretation (11). Moreover, an increase in the production of proinflammatory cytokines, particularly IL-6, occurs with age (18, 24), and includes centenarians (unpublished data). Accordingly, Lp(a) could behave as an acute-phase protein, such as fibrinogen, which also showed a paradoxical increase in healthy centenarians (25).

Another interesting result from this study is the marked difference concerning IL-6 serum levels in randomly selected vs. strictly selected healthy elderly. Indeed, high levels of IL-6 were found in the randomly selected elderly persons, a group that likely includes the most subjects with subclinical diseases. Low serum levels of IL-6 appear to be a reliable marker of healthy status; conversely, high levels of this cytokine can be considered a potent risk factor in the elderly. This finding is in accord with recent data suggesting that high levels of IL-6 are predictors of morbidity in longitudinal studies of cohorts of elderly people (26). From this point of view, the occurrence of healthy centenarians with high levels of IL-6 is another paradox to be added to the increasing list of risk factors that apparently change their biological significance in centenarians (12, 25).

This finding regarding the serum level of Lp(a) in centenarians occurs concomitantly with other interesting changes in serum levels of other major lipids and lipoproteins. Indeed, most centenarians were also characterized by low HDL cholesterol and relatively high triglycerides, two parameters together considered a very strong risk factor for coronary heart disease. On the whole, the levels of plasma lipids and lipoproteins in centenarians apparently are a mixture of characteristics considered either favorable (low total and LDL CT) or unfavorable (low HDL-CT and high TG) for atherosclerosis in young people.

Our data seem to suggest that the physiological and pathological roles of Lp(a) likely change with age despite the relatively stable serum levels of this lipoprotein throughout the entire human life span. These data also support the hypothesis that a continuous reshaping in lipid physiology occurs with age and is likely a critical factor for survival and successful aging.

TABLE 3. *Lipid and lipoprotein levels (mg/dl; x̄ ± SD) in centenarians and controls*

	Centenarians (*n*=75)	<65 years, randomly selected (*n*=114)	>65 years, randomly selected (*n*=73)	>60 years, healthy selected (*n*=30)
CT	189.8 ± 34.9•#	195.9 ± 29.8	228.1 ± 45.1	223.3 ± 33.2
LDL-CT	115.1 ± 27.8•	117.0 ± 26.3	145.5 ± 37.1	n.d.
HDL-CT	49.2 ± 12.9°*	62.2 ± 14.8	54.8 ± 16.5	n.d.
TG	125.3 ± 65.0*	86.1 ± 32.4	139.0 ± 59.6	112.8 ± 48.6

• $P < 0.001$ vs. >65 yrs randomly selected. ° $P < 0.05$ vs. >65 yrs randomly selected. # $P < 0.001$ vs. >60 yrs healthy selected. * $P < 0.001$ vs. <65 yrs randomly selected.

A major consequence of this reshaping is that changes in the serum level of protein, lipids, and lipoproteins that are considered risk factors for atherosclerotic vascular diseases in young people may lose their biological significance and assume a different, unknown role with advanced age. Such a reshaping can have far-reaching consequences for laboratory standard values and, when possible, in therapeutic intervention for the elderly. The results presented here are in line with our general hypothesis that a continuous remodeling develops with time as a result of the continuous adaptation to changes occurring in the body with age in response to internal and external damaging agents (27). FJ

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