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Small dense low-density lipoprotein as biomarker in the elderly

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ABSTRACT

Objectives: Small dense low-density lipoprotein (sdLDL) is atherogenic and associated with atherosclerotic cardiovascular diseases (ASCVD). The aim of this study was to perform the prospective evaluation of sdLDL-c in new ASCVD over 18 years of follow up, and to compare the association of sdLDL-c and conventional lipids and apolipoproteins with ASCVD in the elderly.

Methods: This prospective study included a total of 1770 subjects ≥ 64 years of age with an 18-year follow-up period. The determination of sdLDL-c was measured by a homogenous, selective enzymatic method. Levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG) were determined by enzymatic methods. Apolipoproteins, ApoA1 and ApoB, were analyzed by immunonephelometric methods. Low-density lipoprotein cholesterol (LDL-c) levels were calculated using the Friedewald formula.

Results: According to Pearson's correlation coefficients, sdLDL-c concentration was positively correlated with LDL-c, nonHDL-c, TC and ApoB concentrations. During follow up, sdLDL-c was significantly associated with new ASCVD in men aged 64–76 years in both unadjusted and adjusted Cox regression models. The adjusted hazard ratio (95 % CI) for sdLDL-c was 1.61 (1.13–2.28). No significant associations between sdLDL-c and ASCVD were observed in men aged 77–97 years, nor in women aged 64–79 or 80–100 years.

Conclusions: Lipid and apolipoprotein concentrations of the elderly were high compared to the recommended target values. In addition, lipid and apolipoprotein baseline concentrations were not higher in the ASCVD group than in the control group. Our results indicated that sdLDL-c is as good a marker as ApoB and better than LDL-c.

1. Introduction

Atherosclerotic cardiovascular diseases (ASCVD) cause a significant number of deaths and hospitalizations each year [1]. In the past, atherosclerosis has been described as a metabolic condition in which circulating cholesterol accumulates in the vessel wall; nowadays it is

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established to be a lipid-induced inflammatory disease in the pathogenesis of which chronic low-grade inflammation is a pivotal factor [2]. Extensive lipid accumulation in the intima and the associated inflammatory reactions (cellular and humoral) are the main reasons for the development of atherosclerotic plaque over years or decades [3,4].

Atherosclerotic plaque consists of extracellular lipid particles, necrotic debris, and macrophage foam cells that have accumulated in the intima of the arterial wall, forming a lipid or necrotic core surrounded by a fibrous cap composed of a collagen-rich matrix and smooth muscle cells [4]. Rupture of the fibrous cap of an unstable, advanced plaque leads to exposure of the necrotic core, causing platelet activation and aggregation and thrombus formation [5]. Such catastrophic rupture of atherosclerotic plaque underlies most cardiovascular (CV) events [6].

Genetic factors in the biology of the arterial wall, hypertension, low shear stress, systematic inflammation, smoking, unhealthy diet, physical inactivity, diabetes, and dyslipidemias are known risk factors associated with atherosclerosis [5]. Also aging itself is a well-established risk factor for atherosclerosis [7]. It should be noted that atherosclerotic changes in the arteries may begin early, even in childhood [8]. Therefore, an optimal lipid profile plays an important role in reducing atherosclerosis in people of all ages [9].

In addition to being an important component of cell membranes, cholesterol is also a precursor to steroid hormones and bile acids [10]. A large part of cholesterol is esterified into cholesteryl ester molecules, packaged, and transported in the blood in lipoproteins whose structural proteins are apolipoprotein B (ApoB) or apolipoprotein A1 (ApoA1) [11]. The main fractions of lipoproteins are traditionally defined by their density: high-density (HDL), low-density (LDL), intermediate-density (IDL) and very-low-density (VLDL) [8]. The main trigger initiation of atherogenesis is the retention of low-density lipoprotein cholesterol (LDL-c) and cholesterol-rich ApoB-containing lipoproteins in the arterial wall [12].

The accelerated formation of VLDL particles in the liver increases the number of triglyceride-rich particles circulating in the bloodstream. Cholesterol ester transfer protein modifies particle composition by exchanging triglyceride and cholesterol ester between VLDL and LDL particles and between VLDL and HDL particles. These LDL particles act as a substrate for hepatic lipase, and lipolysis produces small dense low-density lipoprotein (sdLDL) [13]. SdLDL particles are one of the subclasses of LDL particles with smaller particle size and higher density [14,15]. Therefore, sdLDL is estimated to be a more atherogenic lipoprotein due to its higher retention rate into the arterial wall, better penetration into the arterial wall, lower binding affinity to the LDL receptor, lower resistance to oxidative stress, longer plasma half-life, and its susceptibility to biochemical changes, such as glycation and oxygenation [16,17]. In addition to being associated with increased CVD risk [18,19], sdLDL, and especially the levels of sdLDL-c have been associated with type 2 diabetes mellitus, metabolic syndrome, obesity, and low-grade inflammation [20,21].

In the past, several methods, such as ultracentrifugation, nuclear magnetic resonance spectroscopy, and gradient gel electrophoresis, have been used to separate sdLDL particles [22,23]. These methods are laborious and time consuming. The aims of this study were 1) to perform the prospective evaluation of sdLDL-c in new ASCVD over 18 years of follow up and 2) to compare the association of sdLDL-c and conventional lipids and apolipoproteins with ASCVD in the elderly.

2. Material and methods

2.1. Study design and population

The study population was from the Lieto Elderly Study, a longitudinal epidemiological population-based study conducted in the municipality of Lieto in southwestern Finland. All inhabitants born 1933 or earlier were invited between March 1998 and September 1999 to participate in the baseline study (n = 1596). Of those eligible, 273 refused to participate or did not respond, and 63 died before the onset of the present study. A total of 1260 (82 %) persons (age $\geq$ 64 years) participated in the study, including 533 men and 727 women. At baseline, the study protocol included an extensive interview, clinical examinations, and numerous laboratory tests [24].

ASCVD events were defined as ischemic heart disease, cerebral infarctions, stroke, cerebrovascular disease, arterial atherosclerosis, and transient cerebral ischaemic attacks and related syndromes or death to ASCVD (International Classification of Diseases, Tenth Revision (ICD 10) codes I20-I25, I63-I69, I70 and G45). All ASCVD events were identified by obtaining data from the Hospital Discharge Register provided by the National Institute of Health and Welfare in Finland. The data of fatal ASCVD events and mortality were obtained from the Cause of Death Registry, from Statistics of Finland. The data of non-fatal and fatal ASCVD events and mortality from baseline to January 2017 were obtained from national registers and the electronic patient record system using unique personal identity code.

Lipid modifying agents were defined as ATC code C10 and non-ischemic cerebral diseases as ICD 10 codes I63-I69. Diabetes, considered a potential confounder, was defined as a diagnosis of diabetes (ICD 10 codes E10-E14) in the medical records and/or fasting serum glucose level $\geq$ 7 mmol/l measured at baseline. Participants with lipid modifying agents in use (n = 71) and/or non-ischemic cerebral diseases (n = 19) at baseline were excluded. The final study sample consisted of 1170 participants who were followed for 18 years for the incidence of new ASCVD cases.

2.2. Laboratory measurements of lipids and apolipoproteins

Lipid (total cholesterol (TC), HDL-c, LDL-c, triglycerides (TG)) and apolipoprotein (ApoA1 and ApoB) analyses were performed using fresh samples. Blood samples were collected, centrifuged (at 2500 g for 10 min), and plasma aliquots were stored at −70 °C. Analyses of sdLDL-c were performed using stored and previously unthawed samples. In the same sample, we also determined some conventional lipids (TC, HDL-c, and TG) to ensure the shelf life of the samples [25].

TC and TG were measured by enzymatic methods, and HDL-c was measured by a direct enzymatic method using an automated analyzer (Roche Diagnostics, Mannheim, Germany, and Hitachi 917, Hitachi Ltd, Tokyo, Japan). ApoA1 and ApoB were analyzed by immunonephelometric methods using an automated BN II analyzer (Siemens, Marburg, Germany). LDL-c concentrations were calculated according to Friedewald formula [26]. sdLDL-c concentrations were measured by a fully automated homogeneous method (Denka Seiken Co. Ltd, Tokyo, Japan) using an automated Cobas 8000 c702 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Reagents of TC, HDL-c, and TG had been manufactured by Roche and reagents of ApoA1 and ApoB by Siemens. All analyses of lipids and apolipoproteins were performed in the clinical laboratory. The quality of lipid and apolipoprotein measurements was confirmed by quality controls. The intra- and inter-variation coefficients of the control results were <6.0 % in all assays.

The test of sdLDL-c is a multi-step method. In the first step, non-sdLDL lipoproteins are decomposed by a surfactant and sphingomyelinase in the pre-incubation phase of sample and reagent. In the second step, cholesterol is released only from sdLDL particles by the action of another surfactant, and the released cholesterol is then subject to the enzymatic reaction of cholesterol esterase and cholesterol oxidase. The hydrogen peroxide formed in the enzymatic reaction is detected by the Trinder Reaction. The sdLDL assay has been standardized against the ultracentrifugation method, and the FDA has approved for in vitro diagnostics. The manufacturer has announced the limitations of the assay [27].

2.3. Ethics

The Lieto Elderly Study was conducted according to the guidelines of

Table 1
Baseline characteristics of the participants in the Lieto Elderly Study (n = 1170).

Characteristic	Men	Women
	n (%)	
Gender	488 (42)	682 (58)
Diabetes	78 (16)	97 (14)
Hypertension	161 (33)	264 (39)
Current smoking	62 (13)	33 (5)
	Median (IQR), min–max	
Age, years	71.0 (68.0–77.0), 64.0–97.0	73.0 (68.0–79.0), 64.0–100.0
BMI, kg/m^2	26.5 (24.2–29.1), 15.4–41.2	27.0 (23.8–30.1), 14.2–48.9

IQR = Interquartile range.
BMI = Body mass index.

the Declaration of Helsinki. The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol (Diary number 112/1802/2015). Participants provided written informed consent for the study.

2.4. Statistical analyses

Baseline characteristics of the groups were described using mean and standard deviation (SD) for continuous variables and numbers (percentages) for categorical variables. At the first step, the differences in lipids and apolipoproteins between four age quartiles (Q1-Q4) were compared with one-way analysis of variance using Tukey's method in pairwise comparisons and gender differences were tested with two-sample *t*-test. Based on these analyses, further analyses were done in the following groups: Q1-Q3 men (age 64–76 years), Q4 men (age 77–97 years), Q1-Q3 women (age 64–79 years) and Q4 women (age 80–100 years).

Two-sample *t*-test was used to compare the differences in continuous variables (body mass index (BMI), lipids and apolipoproteins) between ASCVD and control groups. The difference between groups in diabetes (categorical variable) was tested with the Chi-squared test. Associations between sdLDL-c and LDL-c, nonHDL-c, TC, and ApoB were examined using Pearson correlation coefficients and scatter plots. Cox proportional hazard regression models were used to determine hazard ratios (HRs) and 95 % CI for the associations of lipids and apolipoproteins with new ASCVD events. P-values < 0.05 were considered statistically significant. Statistical analyses were performed using SAS System for Windows, version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Baseline characteristics

The baseline characteristics of 1170 participants are presented in Table 1. The median age of women was 73.0 years and that of men 71.0 years.

Most conventional lipids and apolipoproteins had a gender difference (data not shown). In the comparisons between the four age quartiles (Q1-Q4), it was found that most lipids and apolipoproteins values in the highest age quartile (Q4) differed from the other quartiles (data not shown), and thus quartiles Q1-Q3 were combined for further analyses leaving the following four groups: Q1-Q3 men (age 64–76 years), Q4 men (age 77–97 years), Q1-Q3 women (age 64–79 years) and Q4 women (age 80–100 years).

The difference in diabetes between ASCVD and control groups was significant in the age group Q1-Q3 in both men (ASCVD group 20 %, control group 8 %, P-value 0.002) and women (ASCVD group 17 %, control group 10 %, P-value 0.025), and Cox regression analyses were adjusted for diabetes in Q1-Q3. Respectively, there were no significant differences in BMI between the ASCVD and control groups.

Table 2
Correlation of sdLDL-c with LDL-c, nonHDL-c, TC and ApoB among the population of the Lieto Elderly Study.

	sdLDL-c	
Laboratory parameter	**Pearson r**	**P-value**
LDL-c	0.64	<0.001
nonHDL-c	0.76	<0.001
TC	0.65	<0.001
ApoB	0.79	<0.001

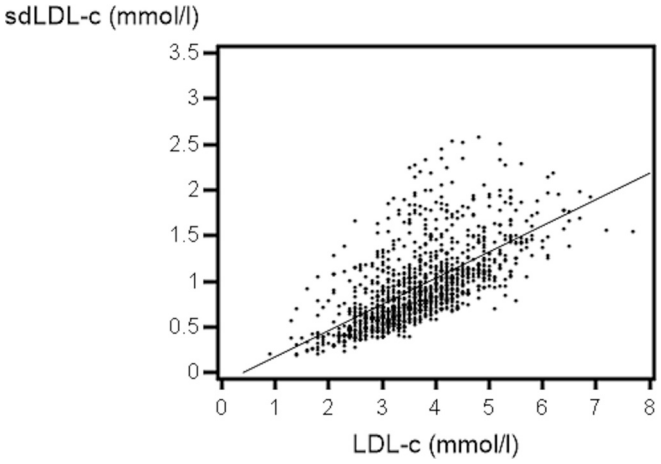

Fig. 1A. Correlation of sdLDL-c with LDL-c among the population of the Lieto Elderly Study. *sdLDL-c: Small dense low-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol.*

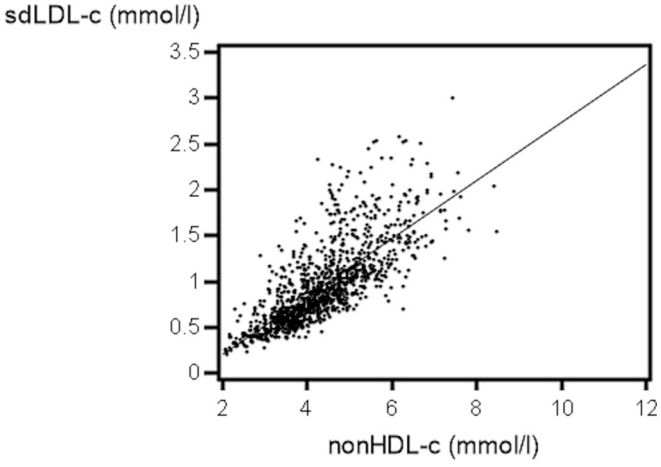

Fig. 1B. Correlation of sdLDL-c with nonHDL-c among the population of the Lieto Elderly Study. *nonHDL-c: Non high-density lipoprotein cholesterol.*

3.2. Associations between lipids and apolipoproteins parameters

According to Pearson's correlation coefficients and scatter plots, sdLDL-c concentration was positively correlated with LDL-c, nonHDL-c, TC and ApoB concentrations (r = 0.64, 0.76, 0.65, 0.79, respectively). The correlations of the sdLDL-c with LDL-c, nonHDL-c, TC, and ApoB concentration are shown in Table 2 and Figs. 1A–D.

3.3. Association of lipids and apolipoproteins with ASCVD risk

The participants were divided into the ASCVD group (n = 314) and a control group (n = 856). The baseline laboratory results of the four

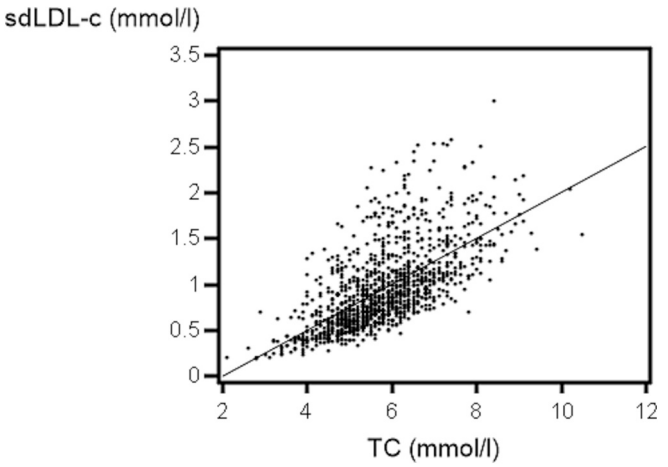

Fig. 1C. Correlation of sdLDL-c with TC among the population of the Lieto Elderly Study. *TC: Total cholesterol.*

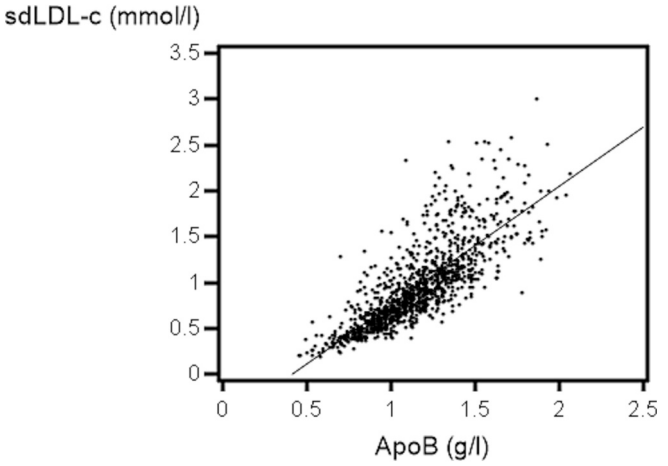

Fig. 1D. Correlation of sdLDL-c with ApoB among the population of the Lieto Elderly Study. *ApoB: Apolipoprotein B.*

groups are shown in Table 3. The baseline results of lipids and apolipoproteins in the ASCVD group do not differ significantly from the results of the control group Q1-Q3 men or women. Correspondingly, the results of lipids and apolipoproteins in the ASCVD group are quite similar to those in the control group Q4 men and women, but small statistical differences can be observed.

The population was followed from baseline for 18 years, and 362 new ASCVD events occurred. These new events occurred in individuals without ASCVD at baseline. Table 4 summarizes the associations of lipids and apolipoproteins with these new ASCVD events. In the group Q1-Q3 men, statistically significant associations were observed with TC, LDL-c, sdLDL-c, nonHDL-c, TG, ApoB, and ApoB/sdLDL-c ratio, in adjusted analyses. In the group Q1-Q3 men, adjusted HRs (95 % CI) for sdLDL-c, ApoB and nonHDL-c were 1.61 (1.13–2.28), 2.18 (1.12–4.25), and 1.30 (1.08–1.57). Conversely, in the group Q1-Q3 women, adjusted analyses did not reveal any significant associations. In the analyses for the Q4 groups, significant associations with ASCVD were observed solely in women for ApoA1 and the ApoB/ApoA1 ratio.

4. Discussion

Conventional lipid measurements remain an important technique for identifying and stratifying the risk of atherosclerotic cardiovascular diseases. The methods for performing lipid analysis are standardized and

Table 3
Baseline characteristics of the different age groups of the participants in the Lieto Elderly Study.

Laboratory parameter	Q1-Q3 men			Q4 men		
	Control (n = 281)	ASCVD (n = 84)	P-value	Control (n = 81)	ASCVD (n = 42)	P-value
TC (mmol/l)	5.48 ± 0.97	5.50 ± 0.97	0.907	5.05 ± 1.03	4.97 ± 1.02	0.686
LDL-c (mmol/l)	3.53 ± 0.87	3.54 ± 0.86	0.939	3.21 ± 0.83	3.17 ± 0.87	0.799
sdLDL-c (mmol/l)	0.98 ± 0.44	1.05 ± 0.45	0.222	0.78 ± 0.41	0.86 ± 0.48	0.281
HDL-c (mmol/l)	1.33 ± 0.32	1.27 ± 0.36	0.182	1.28 ± 0.35	1.14 ± 0.35	0.029
nonHDL-c (mmol/l)	4.16 ± 0.96	4.23 ± 0.93	0.559	3.76 ± 0.94	3.83 ± 0.98	0.712
TG (mmol/l)	1.42 ± 0.72	1.59 ± 0.85	0.097	1.21 ± 0.55	1.45 ± 0.63	0.033
LDL-c/**sdLDL-c**	4.08 ± 1.31	3.87 ± 1.28	0.210	4.78 ± 1.52	4.40 ± 1.59	0.200
TC/HDL-c	4.34 ± 1.17	4.56 ± 1.21	0.129	4.11 ± 1.01	4.67 ± 1.34	0.021
ApoA1 (g/l)	1.47 ± 0.24	1.44 ± 0.25	0.292	1.38 ± 0.27	1.30 ± 0.29	0.098
ApoB (g/l)	1.12 ± 0.26	1.16 ± 0.26	0.159	1.02 ± 0.23	1.07 ± 0.26	0.316
LDL-c/ApoB	3.16 ± 0.33	3.06 ± 0.41	0.057	3.14 ± 0.36	2.98 ± 0.36	0.029
ApoB/ApoA1	0.78 ± 0.23	0.83 ± 0.23	0.080	0.76 ± 0.20	0.86 ± 0.27	0.033
ApoB/**sdLDL-c**	1.28 ± 0.37	1.24 ± 0.38	0.421	1.52 ± 0.46	1.48 ± 0.49	0.627

Laboratory parameter	Q1-Q3 women			Q4 women		
	Control (n = 411)	ASCVD (n = 110)	P-value	Control (n = 83)	ASCVD (n = 78)	P-value
TC (mmol/l)	6.16 ± 1.11	6.04 ± 1.10	0.303	5.64 ± 1.25	5.89 ± 1.28	0.215
LDL-c (mmol/l)	3.90 ± 0.97	3.84 ± 0.94	0.512	3.51 ± 1.05	3.66 ± 1.08	0.373
sdLDL-c (mmol/l)	0.97 ± 0.43	0.94 ± 0.38	0.446	0.80 ± 0.41	1.00 ± 0.52	0.009
HDL-c (mmol/l)	1.60 ± 0.41	1.51 ± 0.39	0.045	1.51 ± 0.44	1.39 ± 0.35	0.073
nonHDL-c (mmol/l)	4.57 ± 1.07	4.53 ± 1.04	0.762	4.13 ± 1.16	4.50 ± 1.21	0.054
TG (mmol/l)	1.46 ± 0.73	1.52 ± 0.62	0.427	1.36 ± 0.53	1.81 ± 0.91	<0.001
LDL-c/**sdLDL-c**	4.45 ± 1.11	4.47 ± 1.13	0.865	4.91 ± 1.39	4.32 ± 1.41	0.010
TC/HDL-c	4.06 ± 1.14	4.20 ± 1.09	0.277	3.98 ± 1.20	4.40 ± 1.40	0.025
ApoA1 (g/l)	1.67 ± 0.28	1.62 ± 0.28	0.087	1.57 ± 0.33	1.49 ± 0.28	0.102
ApoB (g/l)	1.19 ± 0.27	1.19 ± 0.26	0.980	1.09 ± 0.28	1.18 ± 0.32	0.074
LDL-c/ApoB	3.28 ± 0.36	3.23 ± 0.34	0.208	3.21 ± 0.41	3.13 ± 0.41	0.245
ApoB/ApoA1	0.74 ± 0.22	0.76 ± 0.21	0.340	0.72 ± 0.23	0.81 ± 0.23	0.021
ApoB/**sdLDL-c**	1.35 ± 0.32	1.37 ± 0.31	0.453	1.54 ± 0.46	1.35 ± 0.39	0.006

Note: Values are mean ± SD.
Q1-Q3, Q4: Age quartiles; Q1-Q3 men (age 64–76), Q4 men (age 77–97), Q1-Q3 women (age 64–79), Q4 women (age 80–100).
TC: Total cholesterol; LDL-c: Low-density lipoprotein cholesterol; sdLDL-c: Small dense low-density lipoprotein cholesterol; HDL-c: High-density lipoprotein cholesterol; nonHDL-c: Non high-density lipoprotein cholesterol; TG: Triglycerides; ApoA1: Apolipoprotein A1; ApoB: Apolipoprotein B.

Table 4
Unadjusted and adjusted hazard ratios (HRs) and their 95% confidence intervals (CI) of sdLDL-c and conventional lipid profile for ASCVD during the 18-year follow-up among the population of the Lieto Elderly Study.

Laboratory parameter	Q1-Q3 men				Q4 men	
	Unadjusted		Adjusted		Unadjusted	
	HR (95 % Cl)	P-value	HR (95 % Cl)	P-value	HR (95 % Cl)	P-value
TC	1.29 (1.07–1.54)	0.007	1.32 (1.10–1.59)	0.004	0.92 (0.62–1.36)	0.659
LDL-c	1.27 (1.04–1.55)	0.019	1.33 (1.08–1.64)	0.008	0.92 (0.56–1.51)	0.733
sdLDL-c	1.75 (1.24–2.47)	0.002	1.61 (1.13–2.28)	0.008	0.69 (0.26–1.84)	0.464
HDL-c	1.03 (0.55–1.91)	0.932	1.13 (0.62–2.08)	0.684	0.76 (0.30–1.96)	0.575
nonHDL-c	1.28 (1.07–1.53)	0.007	1.30 (1.08–1.57)	0.006	0.94 (0.61–1.45)	0.773
TG	1.37 (1.14–1.66)	0.001	1.20 (1.03–1.54)	0.028	1.11 (0.58–2.14)	0.753
LDL-c/**sdLDL-c**	0.88 (0.77–1.00)	0.049	0.91 (0.79–1.04)	0.153	1.16 (0.89–1.50)	0.279
TC/HDL-c	1.19 (1.03–1.38)	0.021	1.16 (1.00–1.36)	0.056	1.02 (0.73–1.41)	0.922
ApoA1	1.60 (0.81–3.20)	0.179	1.67 (0.83–3.36)	0.148	1.15 (0.35–3.76)	0.816
ApoB	2.04 (1.07–3.90)	0.031	2.18 (1.12–4.25)	0.023	0.68 (0.11–4.10)	0.672
LDL-c/ApoB	1.27 (0.77–2.08)	0.348	1.32 (0.81–2.16)	0.267	0.89 (0.36–2.21)	0.804
ApoB/ApoA1	1.35 (0.66–2.75)	0.412	1.34 (0.64–2.79)	0.434	0.53 (0.09–3.24)	0.493
ApoB/**sdLDL-c**	0.53 (0.33–0.87)	0.012	0.61 (0.37–1.00)	0.048	1.59 (0.69–3.64)	0.276
Laboratory parameter	Q1-Q3 women				Q4 women	
	Unadjusted		Adjusted		Unadjusted	
	HR (95% Cl)	P-value	HR (95% Cl)	P-value	HR (95% Cl)	P-value
TC	1.02 (0.89–1.17)	0.811	1.04 (0.90–1.19)	0.605	1.02 (0.80–1.29)	0.889
LDL-c	1.06 (0.91–1.24)	0.424	1.09 (0.93–1.27)	0.293	0.90 (0.68–1.19)	0.446
sdLDL-c	1.26 (0.88–1.80)	0.205	1.16 (0.83–1.64)	0.386	0.82 (0.37–1.81)	0.624
HDL-c	0.73 (0.48–1.10)	0.136	0.80 (0.53–1.20)	0.281	1.95 (0.94–4.05)	0.071
nonHDL-c	1.06 (0.93–1.22)	0.398	1.07 (0.93–1.23)	0.345	0.92 (0.71–1.20)	0.553
TG	1.08 (0.89–1.29)	0.448	1.02 (0.85–1.23)	0.808	1.07 (0.64–1.82)	0.789
LDL-c/**sdLDL-c**	0.99 (0.86–1.15)	0.915	1.02 (0.89–1.18)	0.771	0.98 (0.79–1.22)	0.880
TC/HDL-c	1.15 (1.01–1.32)	0.037	1.12 (0.99–1.28)	0.072	0.77 (0.58–1.02)	0.064
ApoA1	0.60 (0.33–1.09)	0.092	0.67 (0.37–1.23)	0.185	3.63 (1.43–9.21)	0.007
ApoB	1.37 (0.78–2.42)	0.274	1.36 (0.77–2.39)	0.285	0.57 (0.21–1.54)	0.268
LDL-c/ApoB	1.12 (0.71–1.76)	0.635	1.26 (0.79–2.00)	0.335	1.12 (0.56–2.26)	0.746
ApoB/ApoA1	2.03 (1.00–4.15)	0.052	1.85 (0.92–3.70)	0.084	0.14 (0.04–0.53)	0.004
ApoB/**sdLDL-c**	0.85 (0.52–1.39)	0.523	0.93 (0.59–1.48)	0.756	0.88 (0.44–1.74)	0.703

Group men Q1-Q3 was adjusted for diabetes.
Men Q1-Q3; new ASCVD = 129 and Q4; new ASCVD = 33.
Group women Q1-Q3 was adjusted for diabetes.
Women Q1-Q3; new ASCVD = 161 and Q4; new ASCVD = 39.
Q1-Q3, Q4: Age quartiles; Q1-Q3 men (age 64–76), Q4 men (age 77–97), Q1-Q3 women (age 64–79), Q4 women (age 80–100).
TC: Total cholesterol; LDL-c: Low-density lipoprotein cholesterol; sdLDL-c: small dense low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; nonHDL-c: Non high-density lipoprotein cholesterol; TG: Triglycerides; ApoA1: Apolipoprotein A1; ApoB: Apolipoprotein B.

available in clinical automated laboratories with extensive knowledge in interpreting results and giving clinical advice. However, it has been previously shown that the conventional lipid profile alone cannot identify a significant number of patients at risk for ASCVD events (19). Therefore, it is important to search for biomarkers that could further define subpopulations for therapeutic decisions [27].

According to international guidelines, including the 2017 American Association of Clinical Endocrinologists and American College of Endocrinology (AACE) and the 2019 European Society of Cardiology/European Atherosclerotic Society (ESC/EAS), LDL-c is the primary target of treatment for dyslipidemia, but also ApoB and nonHDL-c were recommended as additional targets [28,29]. Sniderman et al. (2021, 2019) have creditably demonstrated robust evidence for the superiority of ApoB compared to LDL-c and nonHDL-c as a clinical marker of cardiovascular risk [30,31]. Drexel et al. (2021) have previously shown that the LDL-c/ApoB ratio also predicts major cardiovascular events in patients with established atherosclerotic cardiovascular disease [16]. However, the effects of various cardiovascular risk factors in different age groups are primarily based on studies conducted on middle-aged populations [32]. There are also conflicting results regarding the role of lipids and apolipoproteins as risk factors in the elderly (aged >65 years) versus their role in younger people [33].

In this study, we evaluated a fully automated method for measuring plasma sdLDL-c and compared these results with lipids and apolipoproteins such as LDL-c, ApoB and nonHDL-c. In addition, we evaluated the clinical utility of sdLDL in the elderly. This study is one of the first to investigate the novel sdLDL-c method as a risk indicator in the elderly.

Our results showed that lipid and apolipoprotein concentrations in the elderly were quite high in all age groups, although they slightly decreased in the Q4 group. This was thought to be explained by general frailty and reduced protein synthesis [34]. We also found that concentrations of serum albumin and prealbumin were lower in the group Q4 men and women (data not shown). It is noteworthy that there were no significant difference in lipid and apolipoprotein concentrations between the control group and the ASCVD group at baseline. It is important to remember that the most effective way to prevent cardiovascular events is to slow the progression of atherosclerotic plaque with optimal lipid levels throughout life [35].

SdLDL was significantly associated with ASCVD at follow-up based on new atherosclerotic events in the group Q1-Q3 men. The observed association was stronger than that of LDL-c and nonHDL-c and as strong as that of ApoB between the control and ASCVD groups. In the group Q1-Q3 women, no significant association with ASCVD was found. It has been reported that men are more likely to produce more sdLDL than women for a given triglyceride level [36]. The underlying mechanism is due to higher hepatic lipase activity. High triglyceride concentrations have been shown to affect the formation of sdLDL particles and may increase the cardiovascular risk through this pathway [36–38]. In the group Q4 men or women, sdLDL-c, LDL-c, nonHDL-c and ApoB had no significant association between the control and ASCVD groups.

According to our results, the lipid and apolipoprotein concentrations of the elderly were higher than the recommended target values in both the control and ASCVD group. The consensus target values are not suitable for the elderly, because the concentrations are quite high in all groups, including the control group. Limitations of this study were the retrospective nature of the study, only Caucasian racial profile and quite a small number of new ASCVD cases. According to international guidelines, LDL-c, ApoB and nonHDL-c are effective ASCVD risk markers. In our study, sdLDL-c correlated better with ApoB than with LDL-c. Our results indicated that sdLDL-c is as good a marker as ApoB and better than LDL-c in risk assessment of ASCVD in the elderly. In particular, sdLDL-c measurement appears to be an effective way to assess the risk of ASCVD events in the group of men aged 64–76 years. Because the method for the analysis of sdLDL-c in plasma is inexpensive and fully automated, it allows analyzing large sample volumes in routine clinical laboratories [39,40]. This requires further studies that take into account both the biological variability of patient results and the analytical variability of the method.

CRediT authorship contribution statement

Taina T. Katajamäki: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Marja-Kaisa Koivula:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Data curation. **Marika J. Salminen:** Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis, Data curation. **Tero Vahlberg:** Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis, Data curation. **Elisa T.M. Heikkilä:** Writing – review & editing. **Anna M. Viljanen:** Writing – review & editing, Data curation. **Minna K. Löppönen:** Writing – review & editing, Data curation. **Raimo E. Isoaho:** Writing – review & editing, Data curation. **Sirkka-Liisa Kivelä:** Writing – review & editing, Data curation. **Matti Viitanen:** Writing – review & editing, Data curation. **Jorma Viikari:** Writing – review & editing, Data curation. **Laura Viikari:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Data curation. **Kari J. Pulkki:** Writing – review & editing. **Kerttu M. Irjala:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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