

Small Dense LDL Cholesterol and Coronary Heart Disease

Results from the Framingham Offspring Study

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OBJECTIVE: We sought to establish reference values for a new direct assay for small dense LDL cholesterol (sdLDL-C) and to measure sdLDL-C concentrations in patients with established coronary heart disease (CHD) vs controls.

METHODS: Direct LDL-C and sdLDL-C were measured in samples from 3188 male and female participants of the Framingham Offspring Study, including 173 men and 74 women with CHD.

RESULTS: Postmenopausal status and male sex were associated with higher sdLDL-C concentrations ($P < 0.0001$). Cholesterol-lowering medication use was more frequent ($P < 0.0001$) in CHD patients than in controls (46.8% vs 11.4% in men; 35.1% vs 8.8% in women). In men, mean LDL-C was lower in CHD than in controls (3.22

were found in 10.4% and 22.0% of men and in 24.3% and 27.8% of women with CHD, respectively.

CONCLUSIONS: Despite 4-fold greater cholesterol-lowering therapy use, CHD patients had mean LDL-C concentrations above the LDL-C goal of <2.6 mmol/L (<100 mg/dL). Although women with CHD had higher sdLDL-C concentrations than controls, this difference was not seen in men. These findings may explain some of the high residual risk of future CHD events in CHD patients.

Increased plasma LDL cholesterol (LDL-C)⁽¹⁾ concentrations have been shown to be a significant risk factor for coronary heart disease (CHD), and the use of medications that decrease LDL-C concentrations has been shown to reduce heart disease risk⁽¹⁾⁽²⁾. LDL is comprised of a variety of different subfractions that can be separated by ultracentrifugation, gradient gel electrophoresis, nuclear magnetic resonance, and specific precipitation methods⁽³⁾⁽⁴⁾⁽⁵⁾⁽⁶⁾⁽⁷⁾. Gradient gel

Increased plasma LDL cholesterol (LDL-C)⁽¹⁾ concentrations have been shown to be a significant risk factor for coronary heart disease

vs 3.51 mmol/L, $P < 0.0001$), whereas mean sdLDL-C concentrations were similar (0.83 vs 0.84 mmol/L, $P = 0.609$). In women, mean LDL-C was similar in CHD and controls (3.53 vs 3.46 mmol/L, $P = 0.543$), but mean sdLDL-C was higher (0.83 vs 0.68 mmol/L, $P = 0.0015$). The mean percentage of LDL-C as sdLDL-C was higher in both men and women with CHD than controls ($P < 0.01$). Increased LDL-C and sdLDL-C

electrophoresis and nuclear magnetic resonance imaging are semiquantitative methods. For nuclear magnetic resonance analysis a substantial number of assumptions are required to estimate concentrations of LDL subfractions as well as total LDL particle number⁽⁸⁾⁽⁹⁾. Hirano and colleagues have developed a method that uses heparin sodium salt precipitation followed by centrifugation for measuring small dense LDL

Table 1.
Characteristics and plasma lipid concentrations in men and women participating in the FOS (cycle 6) without CHD, diabetes mellitus, cholesterol-lowering medications, and hormone-replacement therapies.^a

Variable	Men (n = 1080)	Women (n = 1012)	P
Age, years	57.1 (9.7)	57.4 (10.5)	0.4851
Body mass index, kg/m ²	28.2 (4.3)	27.1 (5.6)	<0.0001
Total cholesterol, mmol/l	5.21 (0.92)	5.47 (1.02)	<0.0001
Triglycerides, mmol/l	1.29 (0.91–1.81)	1.13 (0.82–1.60)	<0.0001 ^b
HDL-C mmol/l	1.16 (0.32)	1.48 (0.39)	<0.0001
Calculated LDL-C, mmol/l	3.38 (0.82)	3.39 (0.92)	0.7218
Direct LDL-C, mmol/l	3.54 (0.84)	3.49 (0.94)	0.1540
sdLDL-C, mmol/l	0.82 (0.39)	0.67 (0.41)	<0.0001
sdLDL-C jako % LDL-C	22.9 (8.9)	18.6 (8.7)	<0.0001
Large LDL-C mmol/l	2.72 (0.69)	2.82 (0.75)	0.0013
LDL-C <2.6 mmol/L, %	12.2	16.9	0.0024 ^c
LDL-C >4.2 mmol/L, %	23.8	22.7	0.5631 ^c
sdLDL-C <0.5 mmol/L, %	24.1	43.5	<0.0001 ^c
sdLDL-C >1.0 mmol/L, %	26.5	13.6	<0.0001 ^c

^a Values are expressed as mean (SD), median (25th–75th percentile), or percentage.
^b P obtained by χ^2 test.
^c P obtained after log transformation of the data.

Table 2.
Characteristics and plasma lipid concentrations in premenopausal and postmenopausal women participating in the FOS (cycle 6) without CHD, diabetes mellitus, cholesterol-lowering medications, and hormone-replacement therapies.^a

Variable	Premenopausal (n = 313)	Postmenopausal (n = 698)	P
Age, years	46.4 (5.0)	62.4 (8.3)	<0.0001
Body mass index, kg/m ²	26.7 (5.5)	27.2 (5.6)	0.1622
Total cholesterol, mmol/l	5.03 (0.94)	5.68 (0.99)	<0.0001
Triglycerides, mmol/l	1.07 (0.72–1.35)	1.20 (0.89–1.73)	<0.0001 ^b
HDL-C mmol/l	1.47 (0.39)	1.48 (0.39)	0.6906
Calculated LDL-C, mmol/l	3.05 (0.88)	3.55 (0.89)	<0.0001
Direct LDL-C, mmol/l	3.17 (0.91)	3.63 (0.93)	<0.0001
sdLDL-C, mmol/l	0.55 (0.38)	0.72 (0.41)	<0.0001
sdLDL-C jako % LDL-C	17.0 (9.1)	19.3 (8.4)	<0.0001
Large LDL-C mmol/l	2.62 (0.73)	2.92 (0.74)	<0.0001
LDL-C <2.6 mmol/L, %	28.1	11.9	<0.0001 ^c
LDL-C >4.2 mmol/L, %	12.5	27.4	<0.0001 ^c
sdLDL-C <0.5 mmol/L, %	60.7	35.9	<0.0001 ^c
sdLDL-C >1.0 mmol/L, %	8.6	15.6	0.0025 ^c

^a Values are expressed as mean (SD), median (25th–75th percentile), or percentage.
^b P obtained by χ^2 test.
^c P obtained after log transformation of the data.

cholesterol (sdLDL-C) directly. Their results with this method correlated highly with the measurement of cholesterol in sdLDL at a density between 1.044 and 1.063 g/mL as isolated by ultracentrifugation⁽¹⁰⁾⁽¹¹⁾⁽¹²⁾⁽¹³⁾. We used this assay along with direct lipoprotein cholesterol to measure sdLDL concentrations in participants in cycle 6 of the Framingham Offspring Study (FOS). Our goals were to determine reference values for this new assay and identify possible differences in results in CHD cases vs controls.

MATERIALS AND METHODS

STUDY PARTICIPANTS

Participants in the FOS, a long-term community-based prospective observational study of risk factors for CHD, are the offspring and their spouses of the original Framingham Heart Study cohort⁽¹⁴⁾⁽¹⁵⁾⁽¹⁶⁾. During cycle 6 of the FOS (1995–1998) standardized medical history data were collected from all participants and they underwent

a physical examination including measurement of fasting lipid concentrations. All samples were stored in our laboratory at -80°C and were not thawed until we used them for analysis. Selection criteria for the CHD patients at cycle 6 included a history of myocardial infarction, acute coronary insufficiency, or angina pectoris. None of the participants had acute coronary syndrome at the time of the examination. We performed our analyses on all available plasma samples from male and female participants in cycle 6. To determine a reference value for sdLDL-C, we selected male and female participants of FOS (cycle 6) without CHD or diabetes and not taking cholesterol-lowering medications or hormonal replacement therapy (1080 men and 1012 women). We determined sex differences in the healthy population. In healthy women, we also determined differences between premenopausal (n = 313) and postmenopausal (n = 698) women. We also determined differences between CHD cases and controls

(individuals with no evidence of CHD at cycle 6) of each sex. There were 173 male CHD cases and 1335 male controls, and 74 female cases and 1606 female controls. We did not exclude patients on cholesterol-lowering medication from this analysis laboratory measurements.

LABORATORY MARKERS

Total cholesterol, triglyceride, and HDL cholesterol (HDL-C) were determined by standard enzymatic methods. HDL-C was measured after isolation of the HDL supernatant following dextran sulfate magnesium precipitation⁽¹⁷⁾. LDL-C was calculated by using the Friedewald formula⁽¹⁸⁾. For the purposes of this study, we used archived plasma samples that had been frozen at -80°C and never previously thawed for the assessment of direct LDL-C and sdLDL-C by automated standardized enzymatic analysis on a Hitachi 911 automated analyzer. The kits used for these tests (LDL-C and sdLDL-C) were provided by Denka Seiken, Tokyo, Japan. The precipitation reagent (0.1 mL) contained 150 U/mL of heparin-sodium salt (Sigma) and 90 mmol/L of MgCl₂ (Nakarai), and was added to 0.1 mL of plasma, mixed, and incubated for 10 min at 37°C⁽¹⁰⁾⁽¹¹⁾. The samples were then placed in an ice bath for 15 min, and then the precipitate was collected by centrifugation at 21000g for 15 min at 4°C⁽¹⁰⁾⁽¹¹⁾. Aliquots of the supernatant were used for measurement of the cholesterol concentration.

Within- and between-run CVs for the direct LDL-C assay were 0.77% and 1.30% and for sdLDL-C were 4.99% and 4.67%, respectively. We calculated large LDL-C concentrations as: direct LDL-C – sdLDL-C, and we also calculated the percentage of LDL-C as sdLDL-C based on direct measurements. Assays for direct LDL and sdLDL-C have previously been calibrated and directly compared with concentrations obtained after isolation of LDL and sdLDL by ultracentrifugation, and very similar results were obtained⁽¹⁰⁾. When we compared concentrations obtained for direct LDL-C and sdLDL-C in fresh plasma (n = 20) vs concentrations obtained in plasma stored at -80°C for 3 months, we obtained virtually identical results. All laboratory personnel were blinded with regard to the clinical status of study participants. In addition we have not previously observed any effects on plasma measurements of either LDL or HDL particles from use of frozen samples provided the samples were stored at -80°C and never thawed until just before use, and then thawed rapidly at 37°C in a water bath⁽¹⁶⁾⁽¹⁹⁾. Moreover, this issue has been checked and verified by the manufacturer of the sdLDL-C assay, Denka-Seiken. In addition, we received a direct communication

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Table 3.

Means and selected percentiles of direct LDL-C, calculated LDL-C, small dense LDL-C, large LDL-C, and sdLDL-C/LDL-C ratio in participants of the FOS (cycle 6) without CHD, diabetes mellitus, cholesterol-lowering medications, and hormone-replacement therapies, according to sex and menopausal status for women.^a

Variable	Mean		Percentiles				
	n	(ΣΔ)	10.	25.	50.	75.	90.
Direct LDL-C, mmol/l							
Men	1080	3.54 (0.84)	2.49	2.96	3.51	4.10	4.67
Women	1012	3.49 (0.94)	2.32	2.85	3.41	4.08	4.74
Premenopausal	313	3.17 (0.91)	2.15	2.47	3.04	3.74	4.24
Postmenopausal	698	3.63 (0.92)	2.51	3.00	3.50	4.22	4.86
Calculated LDL-C, mmol/l							
Men	1074	3.38 (0.82)	2.35	2.81	3.33	3.93	4.43
Women	1008	3.39 (0.92)	2.29	2.78	3.32	3.90	4.63
Premenopausal	313	3.04 (0.88)	2.08	2.41	2.99	3.54	4.10
Postmenopausal	694	3.55 (0.89)	2.44	2.98	3.47	4.08	4.72
sdLDL-C mmol/l							
Men	1080	0.82 (0.39)	0.36	0.53	0.75	1.05	1.36
Women	1011	0.67 (0.41)	0.27	0.38	0.57	0.86	1.17
Premenopausal	313	0.55 (0.38)	0.23	0.32	0.45	0.65	0.99
Postmenopausal	697	0.72 (0.41)	0.30	0.42	0.64	0.91	1.21
Large LDL-C, mmol/l							
Men	1080	2.72 (0.69)	1.89	2.24	2.70	3.14	3.56
Women	1011	2.83 (0.75)	1.90	2.34	2.75	3.32	3.82
Premenopausal	313	2.62 (0.73)	1.76	2.06	2.59	3.11	3.51
Postmenopausal	697	2.92 (0.74)	1.98	2.44	2.83	3.40	3.92
% of LDL-C as sdLDL-C							
Men	1080	22.9 (9.0)	12.4	16.3	21.8	28.4	35.1
Women	1011	18.6 (8.7)	9.3	12.7	16.8	23.2	29.5
Premenopausal	313	17.0 (9.1)	8.7	11.5	14.6	20.7	27.4
Postmenopausal	697	19.3 (8.4)	9.7	13.5	17.7	23.9	30.4
a Calculated LDL-C calculated by Friedewald formula; % of LDL-C as sdLDL-C calculated by using direct measurements.							

^a Calculated LDL-C calculated by Friedewald formula; % of LDL-C as sdLDL-C calculated by using direct measurements.

from the developer of this assay (Tsutomu Hirano, personal communication, January 23, 2010), who reported that results obtained on EDTA plasma samples frozen at -80°C and never thawed until analysis were virtually identical to those obtained using fresh plasma.

Results of experiments carried out at Denka Seiken, the assay manufacturer, indicated that storage of serum at 4°C and use for the assay within 7 days of sampling resulted in no significant change in the concentrations obtained. Changes in the results began to be seen with sera stored longer than 7 days at 4°C. Studies on frozen sera and plasma were also conducted, and results confirmed that whereas frozen EDTA plasma was suitable for this assay, serum or heparinized plasma were not. The manufacturer generated data on the stability of EDTA plasma over 2 years when stored at -80°C. The manufacturer has noted, as have

we, that quick freezing of the samples following collection is most important for future sdLDL-C measurement using heparin-magnesium precipitation.

All plasma samples from the FOS were collected in

EDTA and were quick frozen, stored at -80°C, and never thawed until used in this investigation.

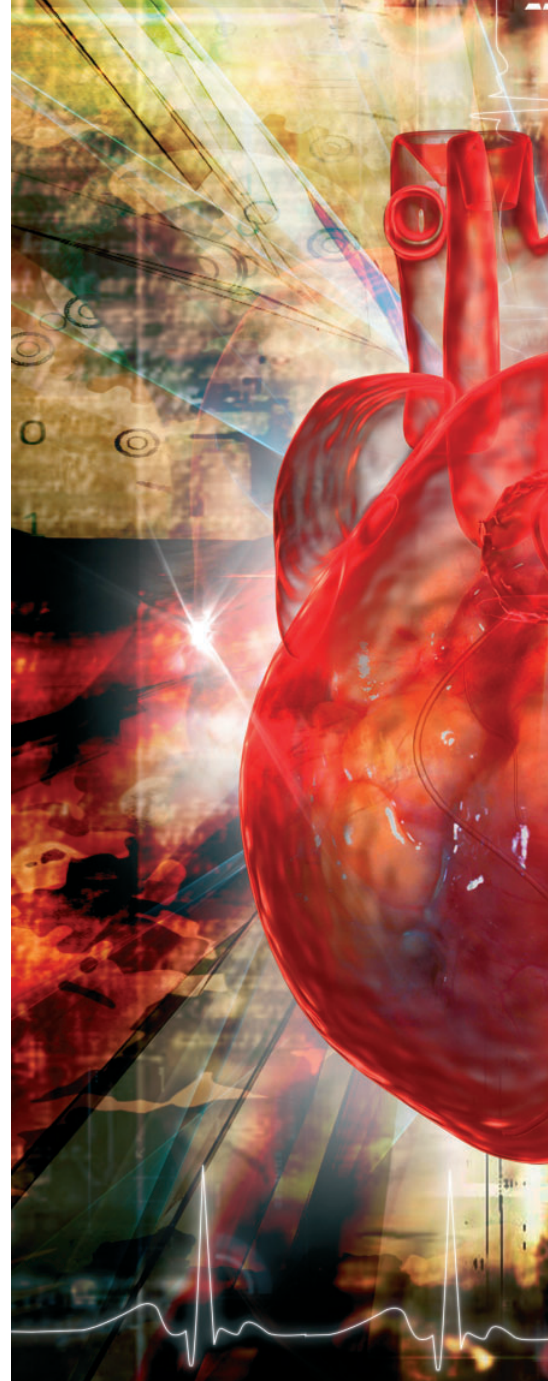
STATISTICAL ANALYSIS

Descriptive statistics, mean (SD) for continuous variables or proportions for categorical variables, were computed for all study variables and all study groups. The distribution of the variables was compared between individuals with or without prevalent CHD, by using 2-sample t-tests (with log-transformed data, if necessary) for continuous variables and χ^2 tests for categorical variables.

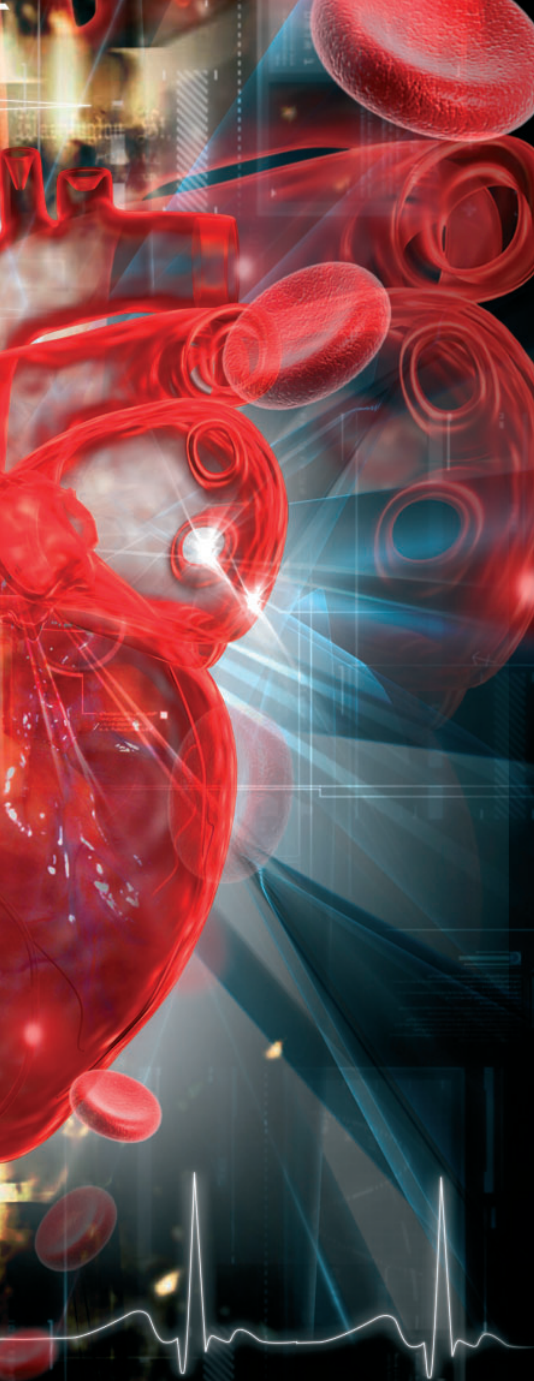
RESULTS

Data on male and female participants in the FOS (cycle 6) without CHD or diabetes and not taking cholesterol-lowering medications or hormone replacement therapy are provided in Table 1. Men and women had similar ages; however, body mass index, waist circumference, systolic and diastolic blood pressure, prevalence of use of antihypertensive treatment and aspirin, and drinking more than 1 alcoholic beverage per week were all significantly higher ($P < 0.0001$) in men than in women (see Table 1 in the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol56/issue6>).

Women had significantly higher concentrations of total cholesterol and HDL-C than men ($P < 0.0001$), whereas men had



significantly higher concentrations of triglyceride and higher total cholesterol/HDL-C ratios than women ($P < 0.0001$). Men and women had similar concentrations of non-HDL-C and LDL-C, but men had significantly higher sdLDL-C concentrations and higher LDL as sdLDL-C percentages than women ($P < 0.0001$). Men were less likely to have an LDL-C concentration in the optimal range of <2.6 mmol/L (<100 mg/dL) as defined by the National Cholesterol Education Program⁽²⁰⁾. Men also were less likely to have sdLDL-C concentrations <0.5 mmol/L (<20 mg/dL) than women. This threshold was based on approximate 25th-percentile concentrations in control individuals. Moreover, men were more likely to have increased sdLDL-C concentrations in excess of 1.0 mmol/L (40 mg/dL) than women. This threshold was based on approximate 75th-percentile concentrations in the control male population. In Japan the cutpoint for an increased sdLDL-C



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Table 4.
Characteristics and plasma lipid concentrations in male FOS (cycle 6) participants with and without CHD.^a

Variable	CHD		P for differences
	(n = 1335)	(n = 173)	
Age, years	58.0 (9.7)	65.3 (7.9)	<0.0001
Body mass index, kg/m ²	28.5 (4.4)	28.7 (4.3)	0.6249
Body mass index >30, %	30.1	31.8	0.6542 ^b
Waist (cm)	101.3 (10.9)	102.4 (10.9)	0.1922
Waist >102 cm, %	42.6	46.2	0.3669 ^b
Systolic Blood Pressure (mmHg)	129.6 (17.1)	129.4 (17.6)	0.8633
Diastolic blood pressure, mmHg	77.7 (9.3)	73.4 (9.5)	<0.0001
Hypertension, %	41.9	66.5	<0.0001 ^b
Hypertensive treatment, %	27.7	58.5	<0.0001 ^b
Taking aspirin regularly, %	31.1	78.0	<0.0001 ^b
Diabetes mellitus, %	11.8	29.5	<0.0001 ^b
Oral glycemic-control drug users, %	4.4	12.2	<0.0001 ^b
On insulin treatment, %	1.4	4.6	0.0029 ^b
β-Blocker users, %	9.4	54.9	<0.0001 ^b
Cigarette smokers, %	14.5	12.7	0.5190 ^b
Alcohol use >1 drink/week, %	54.5	46.8	0.0563 ^b
Cholesterol-lowering drug users, %	11.4	46.8	<0.0001 ^b
Total cholesterol, mmol/L	5.17 (0.93)	4.81 (0.95)	<0.0001
Triglycerides, mmol/L	1.33 (0.94–1.87)	1.43 (1.05–2.11)	0.0127 ^c
HDL-C, mmol/L	1.16 (0.32)	1.04 (0.28)	<0.0001
Total cholesterol/HDL-C ratio	4.66 (3.78–5.64)	4.72 (3.85–5.47)	0.5369 ^c
Non-HDL-C, mmol/L	4.02 (0.93)	3.75 (0.85)	0.0002
Calculated LDL-C, mmol/L	3.32 (0.82)	2.99 (0.78)	<0.0001
Direct LDL-C, mmol/L	3.51 (0.84)	3.22 (0.81)	<0.0001
sdLDL-C, mmol/L	0.84 (0.41)	0.83 (0.39)	0.6094 ^c
% of LDL-C as sdLDL-C	23.7 (9.5)	26.1 (10.0)	0.0019 ^c
Large LDL-C, mmol/L	2.67 (0.70)	2.39 (0.69)	<0.0001
Fasting glucose, mmol/L	5.89 (1.49)	6.45 (1.89)	0.0003
LDL-C < 2.6 mmol/L, %	13.4	22.0	0.0026 ^b
LDL-C >4.2 mmol/L, %	22.4	10.4	0.00033 ^b
sdLDL-C < 0.5 mmol/L, %	23.3	15.0	0.0141 ^b
sdLDL-C >1.0 mmol/L, %	27.6	22.0	0.1157 ^b
Triglyceride >1.7 mmol/L, %	31.1	38.2	0.0607 ^b
Non-HDL-C < 3.4 mmol/L, %	23.0	31.4	0.0151 ^b
Non-HDL-C >4.9 mmol/L, %	15.6	5.3	0.0003 ^b

^a V Values are expressed as mean (SD), median (25th–75th percentile), or percentage.

^b P obtained by χ^2 test.

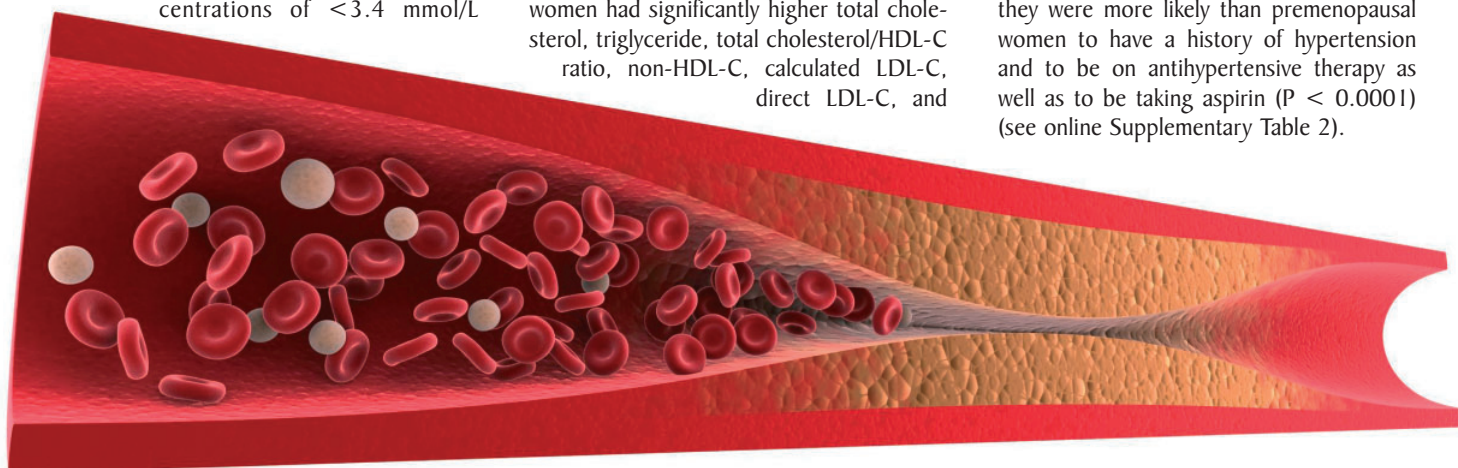
^c P obtained by using log-transformed data.

concentration has been established as >0.9 mmol/L (>35 mg/dL), similar to what we have observed in Framingham. Men also were significantly more likely to have increased total triglyceride concentrations above 1.7 mmol/L (150 mg/dL) and less likely to have non-HDL-C concentrations of <3.4 mmol/L

(130 mg/dL) (see online Supplemental Table 1). These latter cutpoints were identified by the National Cholesterol Education Program Adult Treatment Panel III⁽²⁰⁾.

Information on the differences between premenopausal and postmenopausal women is provided in Table 2. Postmenopausal women had significantly higher total cholesterol, triglyceride, total cholesterol/HDL-C ratio, non-HDL-C, calculated LDL-C, direct LDL-C, and

sdLDL-C concentrations than did premenopausal women ($P < 0.0001$). Postmenopausal women also had significantly higher large LDL-C and higher fasting glucose concentrations. Postmenopausal women had significantly greater waist circumferences and higher systolic blood pressure, and they were more likely than premenopausal women to have a history of hypertension and to be on antihypertensive therapy as well as to be taking aspirin ($P < 0.0001$) (see online Supplementary Table 2).



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Table 5.
Characteristics and plasma lipid concentrations in female FOS (cycle 6) participants with and without CHD.^a

Variable	CHD		P for differences
	(n = 1606)	(n = 74)	
Age, years	58.1 (9.6)	66.1 (8.0)	<0.0001
Body mass index, kg/m ²	27.3 (5.7)	29.1 (5.4)	0.0082
Body mass index 30, %	25.8	35.1	0.0757 ^b
Waist, cm	93.9 (14.8)	101.1 (13.3)	<0.0001
Waist >102 cm, %	26.3	39.7	0.0110 ^b
Systolic blood pressure, mmHg	126.5 (19.6)	140.4 (24.6)	<0.0001
Diastolic blood pressure, mmHg	73.9 (9.1)	73.6 (11.6)	0.8502
Hypertension, %	35.8	83.8	<0.0001 ^b
Hypertensive treatment, %	22.9	73.0	<0.0001 ^b
Taking aspirin regularly, %	19.8	62.2	<0.0001 ^b
Diabetes mellitus, %	8.3	29.7	<0.0001 ^b
Oral glycemic-control drug users, %	2.5	10.8	<0.0001 ^b
On insulin treatment, %	1.2	9.5	<0.0001 ^b
β-Blocker users, %	8.8	47.3	<0.0001 ^b
On estrogen therapy, %	26.3	23.0	0.5307 ^b
Postmenopause, %	76.5	94.6	0.0003 ^b
Cigarette smokers, %	15.5	18.9	0.4322 ^b
Alcohol use >1 drink/week, %	30.5	21.6	0.1035 ^b
Cholesterol-lowering drug users, %	8.8	35.1	<0.0001 ^b
Total cholesterol, mmol/L	5.48 (0.99)	5.58 (1.02)	0.3887
Triglycerides, mmol/L	1.25 (0.88–1.84)	1.53 (1.07–2.05)	0.0030 ^c
HDL-C, mmol/L	1.48 (0.41)	1.40 (0.42)	0.0725
Total cholesterol/HDL-C ratio	3.71 (2.99–4.64)	4.09 (3.47–4.70)	0.0233 ^c
Non-HDL-C, mmol/L	3.98 (1.03)	4.18 (0.99)	0.1177
Calculated LDL-C, mmol/L	3.31 (0.89)	3.33 (0.82)	0.9241
Direct LDL-C, mmol/L	3.46 (0.93)	3.53 (0.87)	0.5430
Small dense LDL-C, mmol/L	0.68 (0.42)	0.83 (0.44)	0.0015 ^c
% of LDL-C as sdLDL-C	19.0 (8.9)	23.6 (12.8)	0.0003 ^c
Large LDL-C, mmol/L	2.79 (0.73)	2.70 (0.78)	0.3420
Fasting glucose, mmol/L	5.54 (1.42)	6.54 (2.48)	0.0009
LDL-C 2.6 mmol/L, %	17.3	13.5	0.4040 ^b
LDL-C 4.2 mmol/L, %	21.8	24.3	0.6068 ^b
sdLDL-C 0.5 mmol/L, %	42.3	27.0	0.0090 ^b
sdLDL-C 1.0 mmol/L, %	15.3	27.8	0.0045 ^b
Triglyceride 1.7 mmol/L, %	29.5	40.5	0.0417 ^b
Non-HDL-C 3.4 mmol/L, %	27.3	13.5	0.0089 ^b
Non-HDL-C 4.9 mmol/L, %	17.5	13.7	0.3999 ^b

^a V Values are expressed as mean (SD), median (25th–75th percentile), or percentage.

^b P obtained by χ^2 test.

^c P obtained by using log-transformed data.

Selected percentile concentrations for direct LDL-C, calculated LDL-C, and sdLDL-C for healthy men and women are presented in Table 3. The Adult Treatment Panel of the National Cholesterol Education Program has selected approximate 75th-percentile concentrations (4.15 mmol/L or 160 mg/dL) for LDL-C as being associated with high CHD risk. The 75th percentile for direct LDL-C was 4.10 mmol/L in men and 4.08 mmol/L in women, with somewhat lower values for calculated LDL-C. The 75th percentile for sdLDL-C was 1.05 mmol/L (40 mg/dL) in men, 0.91 mmol/L (35 mg/dL) in postmenopausal women, and 0.65 mmol/L (25 mg/dL) in premenopausal women. These concentrations are very similar to reference values measured in fresh plasma or serum that we have generated in the US and that have been generated in Japan.

Data comparing male CHD cases and controls are presented in Table 4. Men with CHD had significantly higher mean

age and diastolic blood pressure, and were more likely to have hypertension, to be on therapy for hypertension, to be taking aspirin, to have diabetes, to be on oral glycemic control medication or insulin, to use beta blockers, and to be on cholesterol-lowering medications than controls (P < 0.0001). The percentage of men taking cholesterol-lowering medications was 11.4% in controls and 46.8% in men with CHD. Among all individuals on cholesterol-lowering therapy, 88% were on statin monotherapy, 5% were on statins plus another agent (mainly a fibrate), and the remainder (7%) were on a fibrate, niacin, or resin monotherapy. Very similar distributions were seen in male and female study participants with CHD, as well as in male and female controls on cholesterol-lowering treatment. Therefore, overall, 93% of CHD patients who were receiving cholesterol-lowering medication were receiving some form of statin therapy.

The recommended goal for patients with established CHD, based on the National Cholesterol Education Program, is an LDL-C <100 mg/dL or 2.6 mmol/L⁽²⁰⁾. The mean direct LDL-C in men with CHD was 3.2 mmol/L, and only 22% of men with CHD had an LDL-C below the recommended target. Direct LDL-C concentrations were used for this calculation; however, very similar percentages were obtained when calculated LDL-C was used (e.g., 25%). Male CHD patients had significantly lower mean total cholesterol, but their mean triglyceride concentrations were higher and their mean HDL-C concentrations significantly lower than those in controls, as were their mean calculated LDL-C and direct LDL-C. Despite these results, sdLDL-C did not differ significantly between men with CHD and controls, and men with CHD had higher percentages of LDL-C as sdLDL-C and significantly higher fasting glucose concentrations than controls.

A very similar pattern was observed for the women, but the differences between cases and controls were even greater than for the men (Table 5). Compared with controls, women with CHD were significantly older had a greater mean body mass index and waist circumference and a higher systolic blood pressure. Women with CHD were also more likely than controls to have hypertension and to be on antihypertensive treatment, to be taking aspirin regularly; to be diabetic and be taking medications for diabetes; to be on β blockers, to be on cholesterol-lowering medication, and to be postmenopausal.

Despite the fact that about 4 times as many women were receiving cholesterol-lowering medication in cases than in controls (35.1% vs 8.8%), the total cholesterol concentrations were similar between cases and controls, and the mean triglyceride concentration was significantly higher, as was the mean total cholesterol/HDL-C ratio. Calculated LDL-C and direct LDL-C concentrations were similar, whereas sdLDL-C concentrations were significantly higher in female CHD cases than in controls. Substantially higher numbers of women with CHD had sdLDL-C >1.0 mmol/L compared to controls. Only 13.5% of female CHD cases were at the recommended LDL-C goal of <2.6 mmol/L.

Because there were significant differences between CHD cases and controls in rates of the use of cholesterol-lowering medication, we sought to determine whether the differences we observed in lipid concentrations persisted when we excluded study participants who were on cholesterol-lowering medications, whether they were controls or CHD patients. For men, only 92 CHD cases and 1181 controls remained, and for women 48 cases and 1464 controls remained.

ined. In these analyses no significant differences between cases and controls were observed for either men or women with regard to calculated or direct LDL-C or sdLDL-C concentrations. It should be noted, however, that physicians are less likely to put CHD patients on cholesterol-lowering medications if they are at or close to their LDL-C goal, and the sample size was small. Prospective studies to be carried in this population in the future will provide better insight with regard to the utility of this assay for CHD risk assessment.

DISCUSSION

SdLDL can be assessed by ultracentrifugation, gradient gel electrophoresis, or nuclear magnetic resonance spectroscopy, and studies indicate that patients with CHD have higher concentrations than healthy individuals. However, these methods are labor-intensive and available only in advanced lipid-testing laboratories, and they have not been well standardized. In this study, we evaluated sdLDL-C for the first time in a US population with a method that uses precipitation followed by centrifugation and the measurement of cholesterol concentrations on an automated analyzer. We have generated reference values that agree well with concentrations obtained with fresh plasma.

A major limitation of our study was that we used plasma stored at -80°C , and there is no guarantee that we would have obtained the same results had we used fresh plasma. However, results of comparison studies performed by us and in Japan indicate virtually identical results with the use of fresh vs frozen plasma for sdLDL-C. Moreover, the reference values that we obtained were similar to those obtained in the Japanese population by using fresh plasma or serum samples. Results of case-control studies have demonstrated that CHD cases are more likely than controls to have increased plasma triglyceride and sdLDL concentrations and decreased HDL and large HDL concentrations

(3)(4)(5)(6)(7)(8)(9)(12)(13)(16). The sdLDL-C assay studied here has been applied to Japanese CHD cases and controls, and increased sdLDL-C has been linked to the presence of CHD and to the severity of coronary disease as assessed by angiography⁽¹²⁾⁽¹³⁾. For LDL particles as assessed by gel electrophoresis, the presence of increased sdLDL has been associated with increased CHD risk in the prospective Quebec Cardiovascular Study⁽²¹⁾. In addition, increased total LDL particle number as determined by nuclear magnetic resonance was associated with increased carotid intimal-medial wall thickness in the Multi-Ethnic Study of Atherosclerosis⁽²²⁾. Moreover, increased

sdLDL and total LDL particle numbers predicted recurrent CHD events in the Veteran Affairs HDL Intervention study, and these parameters were favorably affected by gemfibrozil treatment⁽²³⁾. Most recently, the use of a novel ion mobility assessment of lipoprotein subtypes revealed 3 different lipoprotein patterns that have been linked to CHD risk in a Swedish population: (a) increased LDL, (b) decreased HDL, and (c) increased triglycerides and sdLDL and decreased large HDL⁽²⁴⁾.

Many studies have confirmed the link between increased triglycerides, decreased HDL, and increased total and sdLDL, especially in individuals with metabolic syndrome,

visceral adiposity, CRP, triglycerides, and sdLDL-C⁽²⁵⁾. Dietary trans fatty acids also increase sdLDL-C significantly⁽²⁶⁾. Placebo-controlled trials of primary and secondary prevention by use of statin treatment have demonstrated great benefit in CHD risk reduction associated with reductions in LDL-C concentrations, but very substantial residual CHD risk remains⁽¹⁾⁽²⁾. Recently in a large randomized placebo-controlled trial in individuals with normal LDL-C and increased C-reactive protein concentrations, study participants who received rosuvastatin at a dose of 20 mg/day and who got their LDL-C concentrations to <70 mg/dL and their CRP concentrations to

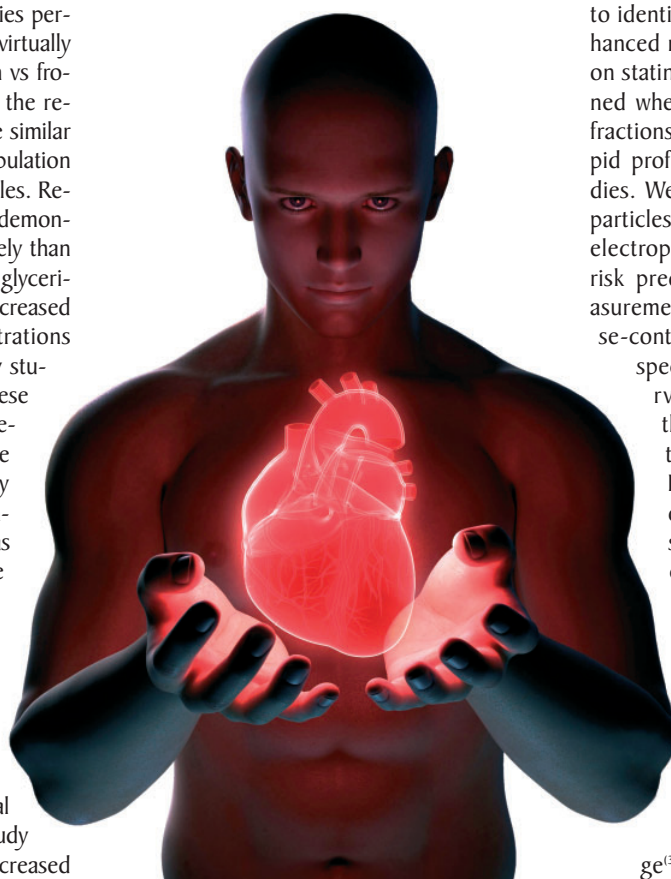
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$\text{me}^{(8)}$. Such individuals also frequently have increased waist circumference and insulin resistance, and increased concentrations of C reactive protein. Giving individuals high fructose diets increases insulin resistance⁽⁸⁾.

Such individuals also frequently have increased waist circumference and insulin resistance, and increased concentrations of C reactive protein. Giving individuals high fructose diets increases insulin resistance⁽⁸⁾.

Investigators have made substantial efforts to subfractionate lipoprotein particles to identify those individuals who retain enhanced residual risk of CHD despite being on statin therapy. It remains to be determined whether assays of lipoprotein particle fractions will be superior to the standard lipid profile in large-scale prospective studies. We have previously shown that HDL particles, as assessed by 2-dimensional gel electrophoresis, provide superior CHD risk prediction compared to routine measurement of HDL-C when tested in a case-control fashion, as well as in the prospective Veteran Affairs HDL Intervention Trial⁽²⁹⁾⁽³⁰⁾.

Treatment with the simvastatin/niacin combination to increase concentrations of large HDL has been shown to be predictive of regression of coronary atherosclerosis⁽³¹⁾. The same may be the case for sdLDL-C vs LDL-C; however, this effect awaits confirmation by prospective data analysis. We have reported that rosuvastatin at a dose of 40 mg/day is even more effective than atorvastatin at 80 mg/day in lowering sdLDL-C by more than 50%, along with lowering LDL-C by a similar percentage⁽³²⁾. Rosuvastatin is also more effective



tive in raising HDL-C and large HDL particles than is atorvastatin⁽³³⁾.

Our data suggest that sdLDL-C has promise as a new test for assessment of heart disease risk. The advantage of this test is that it has excellent reproducibility, and after sample pretreatment, sdLDL-C can be run on high-throughput analyzers, which makes this test much more user-friendly and more applicable than specialized tests such as gradient gel electrophoresis, nuclear magnetic resonance, and gradient ultracentrifugation. These latter tests require shipping samples to specialized laboratories. The sex differences we observed are interesting, as are the differences in sdLDL-C in premenopausal vs postmenopausal women. We have previously reported similar findings for LDL-C and apolipoprotein B (apoB) concentrations in this population⁽³⁴⁾. Aging and menopause are associated with a significant increase in LDL related to delayed clearance⁽³⁵⁾⁽³⁶⁾. Compared with premenopausal women, postmenopausal women also have increased apoB production into VLDLs, which are converted to LDL⁽³⁶⁾. It is well known that CHD risk markedly increases with aging in men and, after menopause, in women, and alterations in LDL clearly contribute to this increased risk⁽²⁰⁾.

Our data also indicate that, despite 4-fold higher cholesterol-lowering medication use (mainly statins) in cases than controls, mean sdLDL-C concentrations were very similar in male cases vs controls, and were significantly higher in female cases than controls. In prospective data from Framingham we have documented that apoB is superior to calculated LDL-C and non-HDL-C in CHD risk prediction, but that the apoB/apoA-I ratio does not provide information about CHD risk that is superior to the total cholesterol/HDL-C ratio⁽³⁷⁾. It remains imperative to carry out prospective analysis to determine whether sdLDL-C is an independent predictor of CHD, and how it compares with apoB concentrations. Ultimately the question remains as to whether clinicians should measure lipoprotein subclasses and apolipoproteins in their patients to optimize prediction of CHD risk. In our view emerging data indicate that these parameters do add information about residual risk, especially in patients with established CHD, but future analyses must be carried out in large prospective cohort studies and intervention studies.

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