



# The Fats of Life

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# Small Dense LDL-Cholesterol in Determining Severe Coronary Atherosclerosis

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## Introduction

Low-density lipoprotein (LDL) particles are heterogeneous with respect to their size, density and lipid composition, and their lipid content chiefly determines the size of LDL particles. High plasma concentration of LDL-cholesterol (LDL-C) is an established major risk factor for coronary heart disease (CHD). On the other hand, it is well accepted that not native LDL particles, but oxidatively-modified LDL particles have the potential to cause foam cell formation, which plays an important role in atherogenesis [1]. Among LDL particles, small dense LDLs have been shown to be easily oxidized *in vitro* and to have lower amounts of lipophilic antioxidants compared with larger LDL particles separated by density-gradient ultracentrifugation [2,3]. In addition, small dense LDL particles have a lower binding affinity for the LDL receptor and have longer clearance time compared with larger LDL. Small dense LDLs penetrate into the arterial wall much more readily and bind to the extracellular matrix more tightly than larger LDL particles [4]. This suggests that small dense LDLs are more pro-atherogenic compared with larger LDLs, and measurement of small dense LDL is useful to detect high risk patients for CHD.

## Serum Lipid Biomarkers for Distinguishing CHD Patients

The Framingham Heart Study [5] has shown that the difference in distribution of LDL-C is very small between CHD patients who did not take any lipid-lowering drugs and the non-diseased population, and about 80%

of CHD patients had LDL-C levels in the same range compared to healthy subjects. The distribution of high-density lipoprotein (HDL)-C concentration, on the other hand, shifts toward lower levels about 10 mg/dL compared to the controls and seemed to be a better predictor of CHD than LDL-C. More than two decades ago, Sniderman and his colleagues [6] compared the cholesterol content and apolipoprotein (apo) B content in LDL fractions separated by ultracentrifugation in 31 patients without and 59 patients with angiographically documented CHD. They showed that LDL-apo B, but not LDL-C, was the better marker to discriminate between patients with and without CHD. Thus, HDL-C or apo B appears to be a better marker to discriminate CHD than LDL-C. Figure 1 compares LDL-C and LDL particle numbers in two subjects. Subject A has a

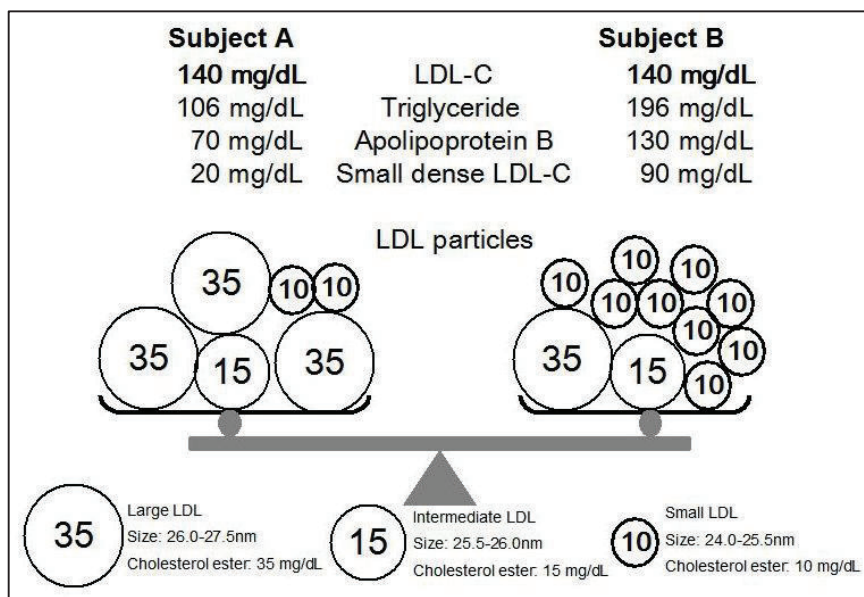


Figure 1. Differences in LDL subclasses between two subjects with the same LDL-C levels. The Number in the circle means the contents of cholesterol esters in each LDL particles.

predominance of cholesterol-rich, large LDL particles, whereas subject B has many small, cholesterol-poor LDL particles, resulting in a marked difference in apo B and small LDL-C concentration. Subject B is characteristic of patients with CHD and/or metabolic syndrome. Thus, increased numbers of small dense LDL particles seems to be a more useful biomarker than LDL-C to detect CHD risk.

### Measurement of Small Dense LDL and Small Dense LDL-Cholesterol

LDL particle size is most often measured by gradient gel electrophoresis using non-denatured 2 to 16% polyacrylamide gel according to the procedure described by Nichols et al [7]. Two distinct LDL size phenotypes, pattern A, large buoyant LDL particles, and pattern B, small dense LDL particles, can be easily separated. Many studies have shown that the predominance of small dense LDL evaluated by this gradient gel electrophoresis is associated with CHD [8-10]. However, this method is not a quantitative assay for small dense LDL-C. We have established a simple and rapid method for measuring small dense LDL-C by heparin magnesium precipitation [11]. Briefly, the precipitation reagent containing heparin and magnesium is added to each serum sample followed by incubation, then the samples are centrifuged, the aggregates are trapped by the filter, and the pass-through fraction is collected for measurement. The clear infranant is then analyzed by direct homogenous LDL-C methodologies.

### Small Dense LDL-C Concentration and CHD

We compared LDL size and small dense LDL-C concentration in 225 consecutive angiographically documented CHD patients who were not receiving any lipid-lowering medication and 95 healthy men, aged 40 to 63 years, and 47 healthy postmenopausal women [12]. The CHD patients were classified into three groups based on the disease type, i.e., acute coronary syndrome (ACS, 73 men and 11 women), including acute myocardial infarction (MI) and unstable angina pectoris; stable CHD (103 men and 20 women), including stable effort angina pectoris and/or prior histories of MI or percutaneous coronary intervention (PCI), and

coronary spastic angina (10 men and 8 women). Similar to our previous study [9], LDL particle size and HDL-C levels were significantly lower in all types of CHD compared with healthy men and women, while LDL-C levels were significantly higher only in patients with ACS (Figure 2). Small dense LDL-C levels were significantly higher in both ACS and stable CHD. On the other hand, large LDL-C, estimated by subtracting the small dense LDL-C concentration from the LDL-C concentration, was somewhat lower in coronary spastic angina and stable CHD.

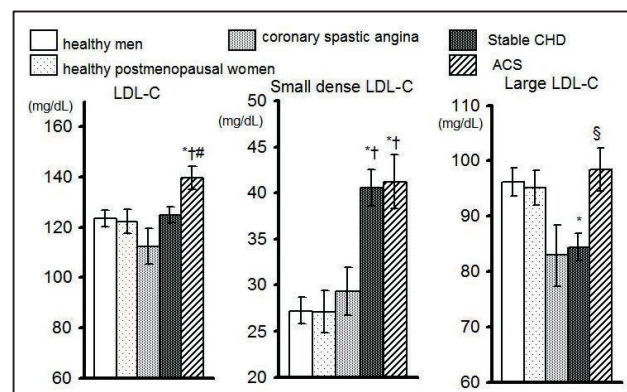
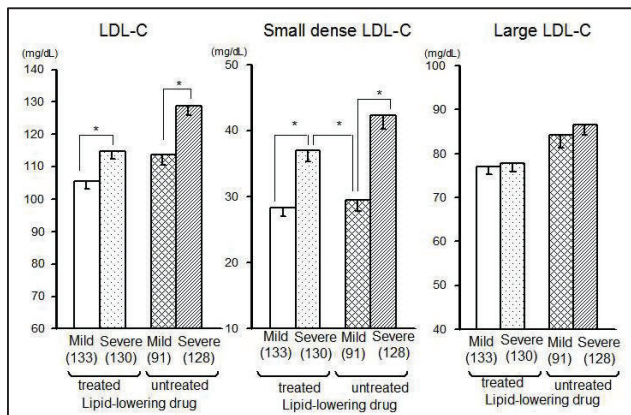


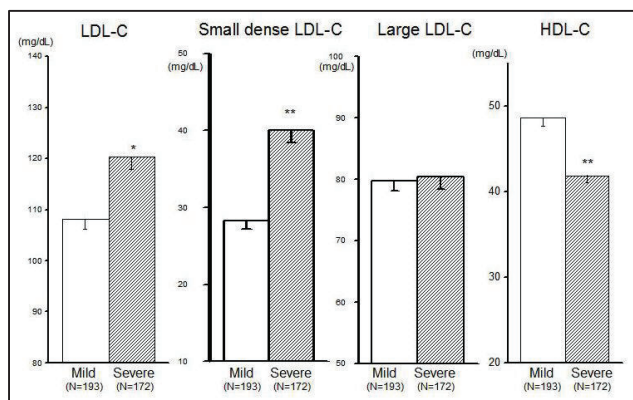
Figure 2. Comparison of LDL-C, small dense LDL-C and large LDL-C among healthy men (N=95), healthy postmenopausal women (N=47), patients with coronary spastic angina (N=18), stable CHD patients (N=123), and ACS patients (N=84). Data are expressed as mean  $\pm$  standard error. \* $P$ <0.05 vs control men, † $P$ <0.05 vs control women, # $P$ <0.05 vs coronary spastic angina, \$ $P$ <0.05 vs stable CHD. Based on reference 12.

The small dense LDL-C concentrations were measured in 482 consecutive stable CHD patients undergoing scheduled coronary angiography [13]. Severe CHD was defined as the presence of stenosis with more than 50% narrowing of the diameter of the left main coronary artery or stenosis with more than 75% narrowing of the diameter in one or more branches of the coronary arteries. Figure 3 shows the comparison of LDL-C, small dense LDL-C, and large LDL-C in patients divided into four groups based on the severity of CHD and the use of lipid-lowering drugs. Among 263 patients on lipid-lowering drugs, 169 male and 53 female patients took statin alone, 23 male and 4 female patients took other lipid-lowering drugs such as



**Figure 3.** Comparison of LDL-C, small dense LDL-C, and large LDL-C levels in patients divided into four groups based on the severity of CHD and the use of lipid-lowering drugs. Data expressed as mean minus standard error. \* $P < 0.0083$  by Bonferroni/Dunn post-hoc test Based on reference 13

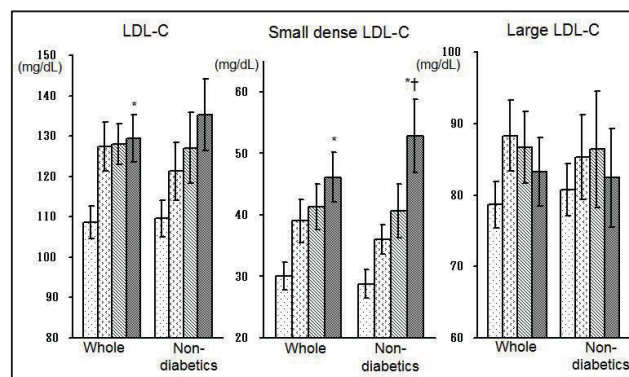
fibrates and eicosapentaenoic acid, and 11 male and 3 female patients were treated with the combination of statin and other lipid-lowering drugs. Patients with severe CHD exhibited significantly higher levels of LDL-C and small dense LDL-C, and similar levels of large LDL-C, irrespective of the use of lipid-lowering drugs. In addition, small dense LDL-C levels were significantly higher in the severe-CHD patients treated with lipid-lowering drugs than in untreated mild-CHD patients, whereas the LDL-C levels were similar and the large LDL-C levels were somewhat higher in unmedicated mild-CHD patients than in medicated severe-CHD patients. Figure 4 compares the LDL-C, small dense LDL-C, large LDL-C, and HDL-C



**Figure 4.** Comparison of LDL-C levels, small dense LDL-C levels, large LDL-C levels, and HDL-C levels between mild and severe CHD among 365 patients with histories of MI and/or PCI. Data expresses as mean minus standard error. \* $P < 0.01$ , \*\* $P < 0.001$  vs the corresponding mild CHD by Student's t-test.

levels between the mild and the severe CHD among 365 patients with histories of MI and/or PCI. Patients with severe CHD exhibited significantly higher levels of LDL-C and small dense LDL-C, significantly lower levels of HDL-C and similar levels of large LDL-C. Therefore, the increases of LDL-C levels in the severe CHD patients compared with the mild CHD patients were chiefly due to those of small dense LDL-C levels, irrespective of the use of lipid-lowering drugs and/or prior histories of MI and PCI.

Table 1 shows the results of logistic regression analysis for determining the severity of CHD among 482 CHD patients. According to our multivariate logistic regression analysis to compare small dense LDL-C with other risk factors, an elevated small dense LDL-C concentration was significantly associated with severe CHD independently of the levels of LDL-C, HDL-C, apo A-1, apo B, non-HDL-C, and HbA1c in stable CHD patients and in CHD patients not taking lipid-lowering agents. Previous case-control studies and prospective studies have shown that non-HDL-C and apo B are both stronger predictors of CHD than LDL-C [14-16]. Our study, on the other hand, identified small dense LDL-C as the most powerful determinant of severe CHD, independent of LDL-C, non-HDL-C and apo B.



**Figure 5.** Associations of LDL-C levels, small dense LDL-C levels, and large LDL-C levels with the severity of coronary atherosclerosis (Quartiles of Gensini score). Quartile 1 (Q1) = Gensini score  $\leq 7.5$ ; quartile 2 (Q2) = 9.0 - 26.0; quartile 3 (Q3) = 27.0 - 49.0; quartile 4 (Q4) = the score  $\geq 52.0$ . The overall population included 36, 36, 34, and 35 patients in Q1, Q2, Q3, and Q4, respectively. \* $P < 0.05$  vs Q1, + $P < 0.05$  vs Q2 by Tukey-Kramer post-hoc test. Based on reference 12

Table 1

Logistic regression analysis for severe CHD

Univariate logistic regression analysis	Overall CHD (N=482)			Non-lipid-lowering group (N=219)		
	Odds	95% CI	p	Odds	95% CI	p
age	1.009	0.992 - 1.026	NS	1.007	0.982 - 1.033	NS
Hypertension	1.290	0.828 - 2.008	NS	1.300	0.672 - 2.514	NS
Diabetes	1.470	1.018 - 2.123	0.0396	1.411	0.813 - 2.449	NS
LDL-C	1.015	1.008 - 1.021	<0.0001	1.014	1.005 - 1.023	0.0015
Small dense LDL-C	1.034	1.022 - 1.045	<0.0001	1.034	1.018 - 1.051	<0.0001
Non-HDL-C	1.010	1.004 - 1.016	0.0007	1.009	1.001 - 1.017	0.0269
HDL-C	0.963	0.949 - 0.978	<0.0001	0.966	0.944 - 0.987	0.0022
Triglyceride	1.003	1.000 - 1.006	0.0241	1.002	0.999 - 1.006	NS
Apo A-1	0.978	0.970 - 0.986	<0.0001	0.981	0.970 - 0.993	0.0020
Apo B	1.022	1.012 - 1.031	<0.0001	1.022	1.009 - 1.036	0.0008
HbA1c	1.283	1.096 - 1.501	0.0019	1.403	1.084 - 1.817	0.0100
hs-CRP	1.304	0.926 - 1.835	NS	1.613	0.932 - 2.792	NS

Multivariate logistic regression analysis	Overall CHD (N=482)			Non-lipid-lowering group (N=219)		
	Odds	95% CI	p	Odds	95% CI	p
LDL-C	1.009	0.994 - 1.024	NS	1.010	0.990 - 1.031	NS
Small dense LDL-C	1.022	1.005 - 1.039	0.0092	1.027	1.003 - 1.051	0.0255
Non-HDL-C	0.989	0.973 - 1.006	NS	0.978	0.952 - 1.006	NS
HDL-C	1.026	0.983 - 1.071	NS	0.999	0.944 - 1.057	NS
Triglyceride	1.001	0.997 - 1.005	NS	-	-	-
Apo A-1	0.974	0.953 - 0.996	0.0215	0.992	0.964 - 1.021	NS
Apo B	1.013	0.985 - 1.041	NS	1.025	0.984 - 1.069	NS
Hb A1c	1.213	1.024 - 1.437	0.0253	1.204	0.952 - 1.006	NS

95%CI = 95% confidence interval, NS = no statistical significance

### Small Dense LDL-C Concentration and Severity of Coronary Atherosclerosis

The severity of coronary atherosclerosis was estimated by calculating the Gensini score by coronary arteriography, an established method for grading coronary stenosis. Small dense LDL-C gradually increased as the Gensini score increased, while large LDL-C did not differ among the different quartiles of Gensini score (Figure 5) [12]. This trend of increased small dense LDL-C along with the increased Gensini score was even more pronounced when the diabetic patients were excluded. Furthermore, the Gensini scores significantly increased along

with elevated levels of small dense LDL-C among stable CHD patients untreated with lipid-lowering drugs (Figure 6). On the other hand, decreases in HDL-C levels are significantly associated with

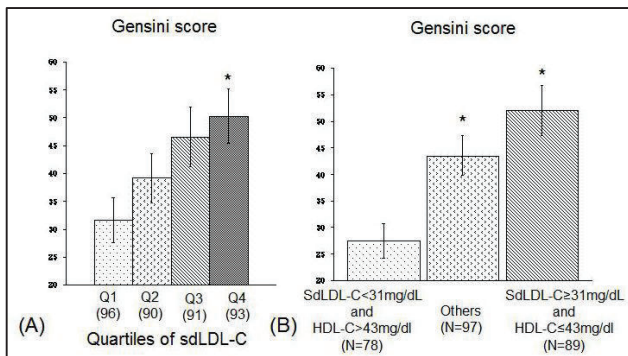


Figure 6. Associations of Gensini score with small dense LDL-C levels, and small dense LDL-C and HDL-C levels. Quartiles of small dense LDL-C. Quartile 1 (Q1) = small dense LDL-C ≤ 20.5; quartile 2 (Q2) = 20.6 - 31.4; quartile 3 (Q3) = 31.6 - 48.0; quartile 4 (Q4) = the score ≥ 48.5. The population included 96, 90, 91, and 93 patients in Q1, Q2, Q3, and Q4, respectively. \*P<0.05 vs Q1 by Tukey-Kramer post-hoc test. (B) Median levels of small dense LDL-C (sdLDL-C) and HDL-C were 31 mg/dL and 43 mg/dL, respectively. Associations of Gensini score and metabolic dyslipidemia (high small dense LDL-C and low HDL-C) in subjects untreated with lipid-lowering agents. \*P<0.05 vs subjects with low small dense LDL-C and high HDL-C by Tukey-Kramer post-hoc test. Based on reference 9 and 13.

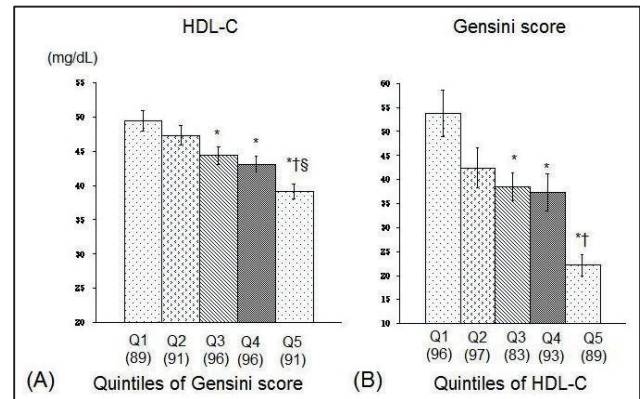


Figure 7. Associations of Gensini score with HDL-C levels. (A) Quintiles of Gensini score. Quintile 1 (Q1) = Gensini score ≤ 9.0; quintile 2 (Q2) = 9.5 - 21.0; quintile 3 (Q3) = 21.5 - 39.5; quintile 4 (Q4) = 40 - 62; quintile 5 (Q5) ≥ 63. The population included 89, 91, 96, 96, and 91 patients in Q1, Q2, Q3, and Q4, respectively. \*P<0.05 vs Q1, †P<0.05 vs Q2, §P<0.05 vs Q3, by Tukey-Kramer post-hoc test. (B) Quintiles of HDL-C. Quintile 1 (Q1) = HDL-C ≤ 34; quintile 2 (Q2) = 35 - 40; quintile 3 (Q3) = 41 - 46; quintile 4 (Q4) = 47 - 54; quintile 5 (Q5) ≥ 55. The population included 96, 97, 83, 93, and 89 patients in Q1, Q2, Q3, and Q4, respectively. \*P<0.05 vs Q1, †P<0.05 vs Q2 by Tukey-Kramer post-hoc test. Based on reference 13.

increases in Gensini scores (Figure 7). The Gensini scores were significantly higher in CHD patients with higher levels of small dense LDL-C and lower levels of HDL-C compared with CHD patients with lower levels of small dense LDL-C and higher levels of HDL-C (Figure 6).

Figure 8 demonstrates the correlation between LDL-C or small dense LDL-C and HDL-C levels among 591 men and 180 women who did not take any lipid-lowering drugs. The LDL-C concentration was strongly correlated with small dense LDL-C ( $r = 0.604, P < 0.0001$ ). Small dense LDL-C, but not total LDL-C was significantly correlated with HDL-C levels ( $r = -0.384, P < 0.0001$ ). These data suggest that high levels of small dense LDL-C correlate with both atherogenic LDL and metabolic dyslipidemia such as increased triglyceride-rich lipoproteins and decreased HDL-C.

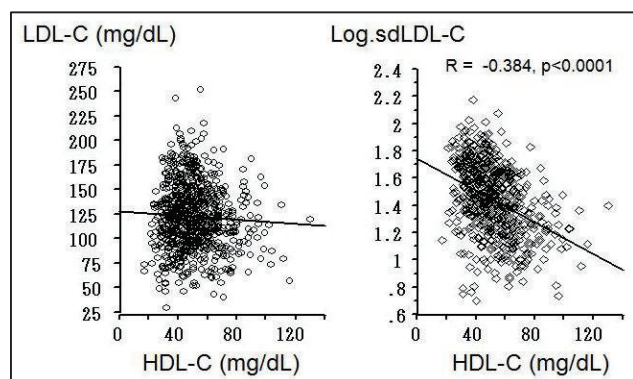


Figure 8. The correlation between LDL-C or small dense LDL-C and HDL-C levels among 591 men and 180 women who did not take any lipid-lowering drugs.

### Conclusion

The increase in LDL-C levels in the clinically and angiographically-graded severe CHD patients compared with patients without severe coronary atherosclerosis were chiefly due to small dense LDL particles. Therefore, elevated small dense LDL-C concentration is a very promising risk marker to detect the progression of coronary atherosclerosis, to predict cardiovascular events, and to assess metabolic dyslipidemia.

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