3.2. Homogeneous assay precision

The inter-assay CVs ranged from 0.18% to 0.47% and the intra-assay CVs ranged from 0.10% to 0.39% for 2 pooled sera with low and high LDL-C levels. The maximum total CV was 0.54%, which is below the NCEP target value (4%) (Table S6).

3.3. Relationship between LDL-C (H) and LCL-C (BQ)

For each sample, we used averages of the triplicate determinations of LDL-C (H). In non-diseased subjects, the median %differences between LDL-C (H) and LDL-(BQ) were close to zero in all the reagents. Only a few results exceeded NCEP total error goals (12%) in most reagents except when Reagent-D and Reagent-G were used (Fig. 1, the left panel).

In diseased subjects, Reagent-C, Reagent-D, Reagent-E, and Reagent-F showed marked discordant results (Fig. 1, the right upper panel). Such discrepancies were found mostly in the positive % bias area where LDL-C (H) was higher than LDL-C (BQ). Note that samples of type I and type III hyperlipidemic subjects caused marked discrepancies for most reagents. Without these data, % bias was <20% in all samples for Reagent-A, Reagent-H, Reagent-I, and Reagent-K (Table S7). We conducted the same analysis for subjects with TG levels <6.78 mmol/L (600 mg/dL) or <4.52 mmol/L. In these subgroups, discordant results with %bias >12% decreased for Reagent-C, Reagent-E, and Reagent-F, whereas they were found

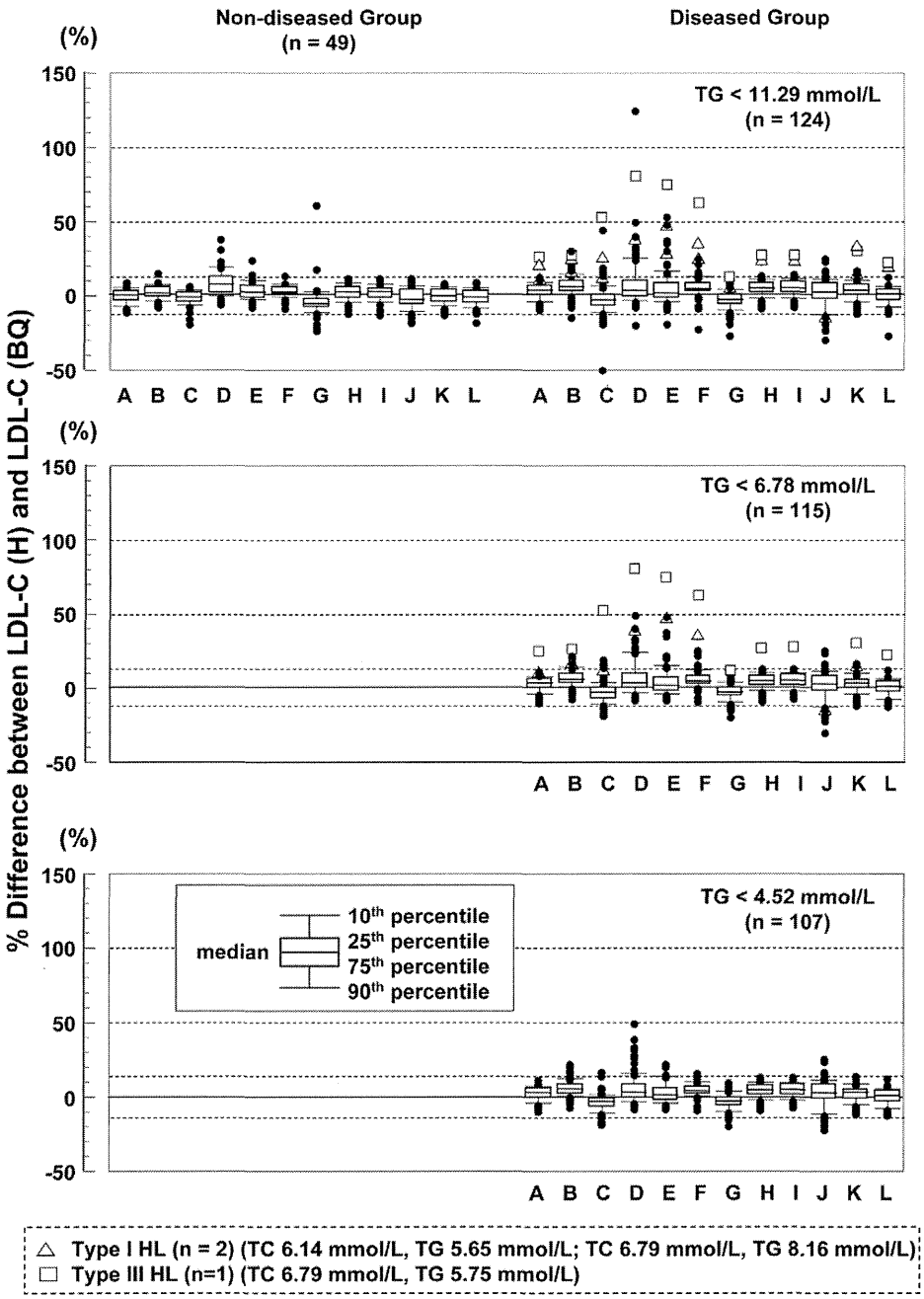

Fig. 1. Box-and-wisker plots of the percent difference between LDL-C (H) and LDL-C (BQ) for non-diseased and diseased subjects Percent differences were calculated from the first determined value of each reagent by the following equation: [LDL-C (H)−LDL-C (BQ)] × 100/LDL-C (BQ). Two samples from diseased subjects were excluded for Ortho's reagent because their values were below lower determination limit. A, Denka Seiken; B, Wako; C, Sysmex,; D, Serotec; E, Fureiya; F, Kyowa; G, Toyobo; H, Shino-Test; I, Sekisui Medical; J, Ortho Clinical Diagnostics; K, Siemens Healthcare Diagnostics; L, Beckman Coulter (See Supplemental Table S1 for detail).

across all TG levels for Reagent-D and Reagent-J (Fig. 1, the middle and lower panels).

Scatter plots showed that LDL-C (H) for each reagent exhibited a strong linear correlation with LDL-C (BQ) in both non-diseased and diseased subjects. The slopes of the linear regression lines were close to 1.0 for all but Reagent-J (Fig. 2, Table S8). In samples with low LDL-C and high TG levels, LDL-C (H) was often higher than LDL-C (BQ) especially for Reagent-C, Reagent-D, Reagent-E, and Reagent-F. In particular, type I and III hyperlipidemia exhibited discordant results for all but Reagent-G and Reagent-J (Fig. 2).

The % bias negatively correlated with LDL-C (BQ) for Reagent-B and Reagent-E, but positively correlated for Reagent-J (Fig. 2). Many discordant results were found across TG levels for Reagent-D, but were limited for Reagent-C and Reagent-F in samples with low LDL-C and high TG levels.

In addition to % bias plots, we drew the Bland-Altman plots where *X*-axis represents the mean values of LDL-C (H) and LDL-C (BQ), and *Y*-axis represents the absolute difference between LDL-C (H) and LDL-C (BQ) (Fig. 3). Reagents-C, Reagent-D, Reagent-E and Reagent-F exhibited greater absolute differences between LDL-C (H) and LDL-C (BQ) than the others. In the patients with LDL-C>6.0 mmol/L, the absolute bias varied among the reagents.

3.4. Total error for single measurements

We carried out this analysis using the first values in triplicate determinations of LDL-C (H). In non-diseased subjects, all but Reagent-D fulfilled the requirement in more than 90% of the samples (Table 1), and 8 reagents reached the 95% level. In diseased subjects, only one-third of reagents reached the 90% agreement. These percentages increased when we re-analyzed samples with TG levels < 6.78 mmol/L or <4.52 mmol/L.

3.5. Error component analysis

In non-diseased subjects, 9 reagents fulfilled the NCEP criteria. The inter-assay CVs (CV_b) were not more than 1.0% in 8 reagents (Table 2). The maximum intra-assay CV (CV_e) was only 1.1%. Although CV_d values were greater than CV_b and CV_e values, CV_d values ranged from 3.2% to 7.0%. CV_t values were very similar to CV_d values. The mean % bias values were less than ±1.0% except for Reagent-D. Although we excluded one extreme outlier for Reagent-G (Fig. 2, marked with a dotted circle), it did not meet the NCEP criteria.

In diseased subjects, the results were worse than those in non-diseased subjects, mainly because of sample-dependent discrepancies between LDL-C (H) and LDL-C (BQ). CV_e values were less than 1.0% except for Reagent-J. However, CV_d, which primarily determines CV_t, showed marked differences among the reagents, ranging from 4.7% to 10.0%. The mean % bias values were less than ±1.0% in half of the reagents and 1.6% at the most. Consequently, 4 reagents fulfilled the NCEP criteria.

3.6. Comparisons between LDL-C (H) and LDL-C (F)

We classified samples with TG levels <4.52 mmol/L into a low TG [<2.26 mmol/L (200 mg/dL)] and a moderately elevated TG (2.26 ≤ TG < 4.52 mmol/L) groups [17]. LDL-C (F) levels were calculated regardless of the fasting interval.

In the low TG group, LDL-C (F) reflected LDL-C (BQ) more accurately than LDL-C (H) (Table S9). For all reagents, the coefficients of determination (R^2) between LDL-C (BQ) and either LDL-C (H) or LDL-C (F) were 0.99. Six LDL-C (H) and 2 LDL-C (F) exhibited significant positive proportional bias against LDL-C (BQ). Furthermore, 6 LDL-C (H) and 3 LDL-C (F) exhibited significant positive fixed bias against LDL-C (BQ). As compared with CVD risk classification with LDL-C (BQ), we misclassified 11%–23% of subjects with LDL-C (H), and 6%–19% of the subjects with LDL-C (F).

In contrast, in the moderately elevated TG group, LDL-C (H) reflected LDL-C (BQ) more accurately than LDL-C (F). R^2 values between LDL-C (BQ) and either LDL-C (H) or LDL-C (F) were 0.99, although they were 0.98 for Reagent-C and Reagent-E for LDL-C (F). Seven LDL-C (H) and 11 LDL-C (F) exhibited significant positive proportional bias against LDL-C (BQ). Furthermore, 5 LDL-C (H) and all LDL-C (F) except for Reagent-A were positive for a fixed bias. We misclassified 14%–34% of subjects with LDL-C (H), and 28%–45% of subjects with LDL-C (F).

4. Discussion

This study indicates that LDL-C (H) are generally in good agreement with LDL-C (BQ) for non-diseased subjects, except for with Reagent-D and Reagent-J, but the performances of Reagent-C, Regent-D, Reagent-E, Reagent-F, and Reagent-J are not as satisfactory for diseased subjects. We found that 8 reagents met the NCEP total error requirement for non-diseased subjects (Table 1). With Reagent-C, Reagent-D, Reagent-E, and Reagent-F LDL-C (H) were higher than LDL-C (BQ) for diseased subjects, especially those with hypertriglyceridemia (Fig. 1).

Since there is no reliable method to determine the "correct" LDL-C levels in pathological conditions, we used LDL-C (BQ) as a reference for evaluation of LDL-C (H). Subjects with rare dyslipidemia and cholestatic liver disease were excluded because they have very low LDL-C levels or abnormal LDLs [12–14]. Similar to Miller's study, our study showed that diseased subjects had greater discordance between LDL-C (H) and LDL-C (BQ) than non-diseased subjects [12]. Individual homogeneous assays have different determination principles [20] (Table S1) and different reactivities to lipoproteins [21–25].

In this study, the accuracies of Reagent-C, Reagent-D, Reagent-E, and Reagent-F were more susceptible to hypertriglyceridemic conditions (Fig. 1). Except for subjects with type I and III hyperlipidemia, Reagent-A, Reagent-H, Reagent-I, and Reagent-K were marginally affected by hypertriglyceridemia (Fig. 1). Yamashita et al determined LDL-C levels in dyslipidemic subjects by a homogeneous assay (Sekisui) and ultracentrifugation. LDL-C (H) showed a positive mean % bias against LDL-C determined by ultracentrifugation in any WHO phenotype. Difference in LDL-C between both methods was the greatest in subjects with type I hyperlipidemia (37.9%), and ranged from 5.2% to 20.2% for other types of dyslipidemia [24]. Subjects with type I hyperlipidemia had very low LDL-C levels, yielding high % bias because of the small denominators. To avoid potential misinterpretation of % bias plots, we also drew the Bland-Altman plots using the absolute LDL-C values. They showed that differences were overemphasized in low LDL-C samples especially in Reagent-A, Reagent-F, Reagent-H, Reagent-I, Reagent-K, and Reagent-L, and that Reagent-C, Reagent-D, Reagent-E, and Reagent-F were more susceptible to hypertriglyceridemia than the others (Fig. 3). All homogeneous assays have passed the "LDL-C certification protocol for manufacturers" provided by the CDC [15]. Since discrepancies between LDL-C (H) and LDL-C (BQ) were found mostly in hypertriglyceridemic subjects, the performance of homogeneous assays should be examined with samples including hypertriglyceridemic sera before approval.

Although TG levels increase in the postprandial state [26], postprandial samples were not the main factor for discordance between LDL-C (H) and LDL-C (BQ) for most reagents. Nearly half of our diseased subjects were in the postprandial state. All reagents except for Reagent-D had a few postprandial samples whose % bias exceeded 20% (Table S7). This may be beneficial for

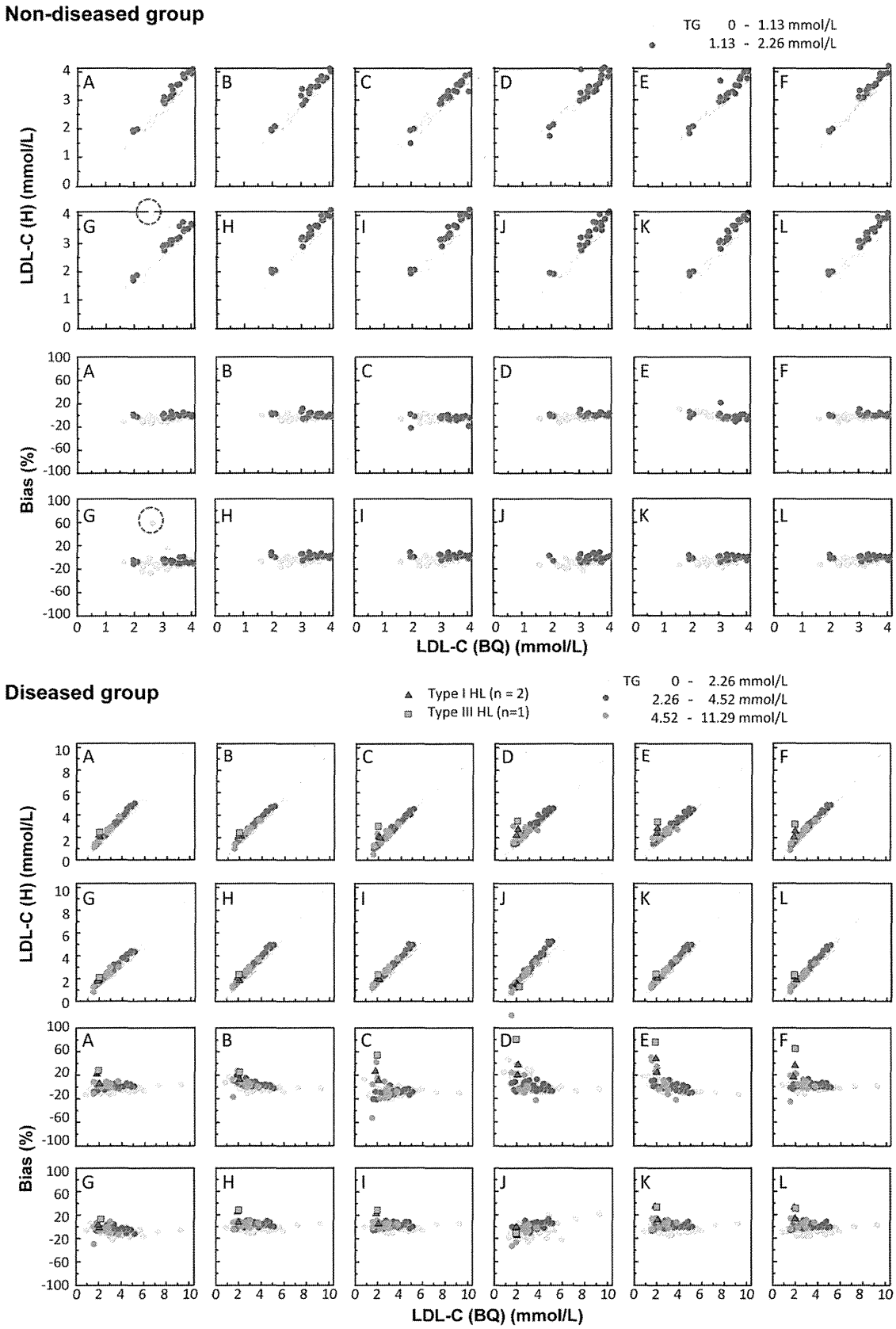

Fig. 2. Relationships of LDL-C (H) and %bias with LDL-C (BQ) In non-diseased and diseased groups, the upper 2 rows indicate scatter plots of LDL-C (H) versus LDL-C (BQ), while the lower 2 rows indicate %bias plots of %differences between LDL-C (H) and LDL-C (BQ) versus LDL-C (BQ).

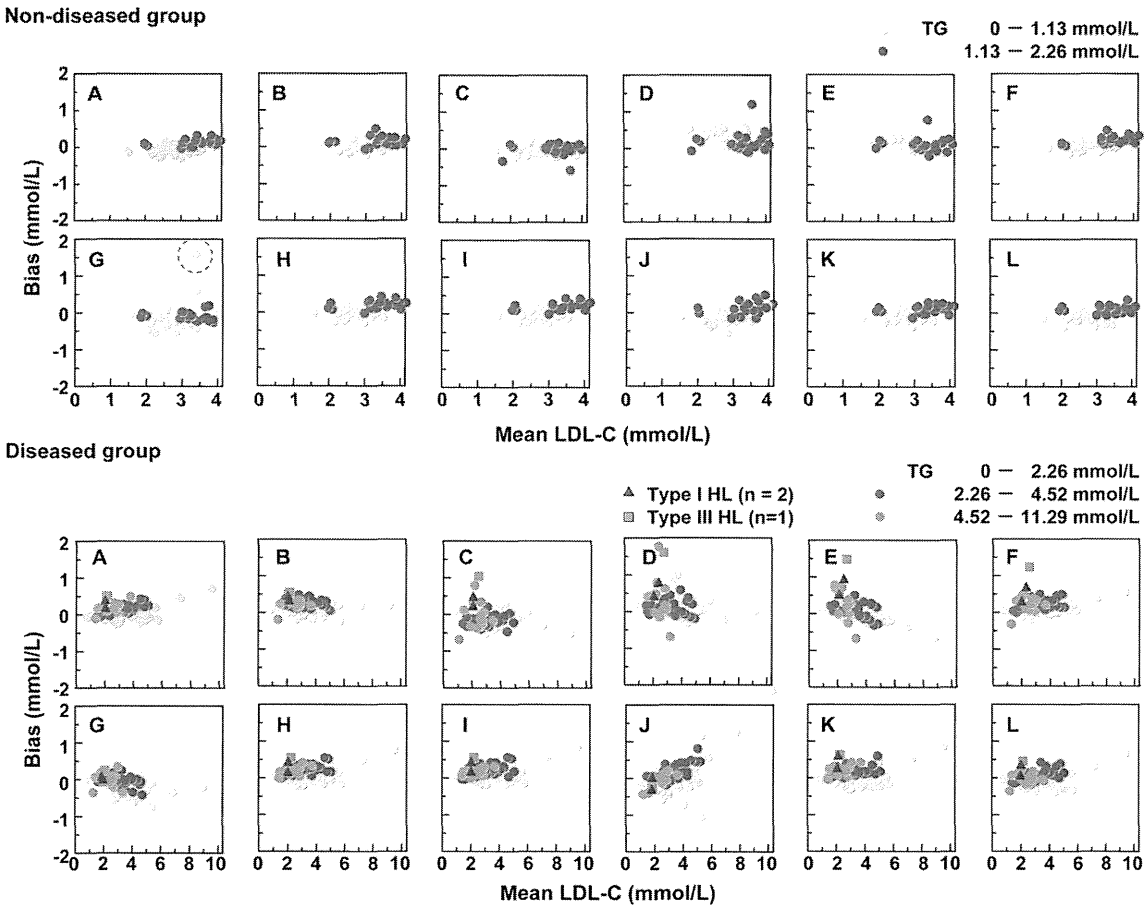

Fig. 3. Bland-Altman plots of LDL-C (H) and LDL-C (BQ). The absolute bias was plotted against mean values of LDL-C (H) and LDL-C (BQ).

studies that recruit community residents or subjects with acute coronary syndrome because they may not be fasted before blood sampling [27,28]. A recent meta-analysis, using 8 long-term prospective studies, recruited 44,234 participants without initial CVD. The hazard ratio for coronary heart disease was calculated for 1-SD higher LDL-C (H). After adjustment for conventional risk factors, the hazard ratio was 1.38 (95% CI, 1.09–1.73) [29]. Meanwhile, Mora et al failed to show a relationship between LDL-C (H) and CVD in their 11-year study involving 27,331 healthy women [30]. Unfortunately, they used frozen serum for LDL-C measurement by a homogeneous assay. The accuracy of LDL-C (H) has not been verified for frozen samples. More studies are required to clarify whether we can predict a risk of CVD with LDL-C (H).

For future standardization of LDL-C (H), we have to reduce not only the diversity in reactivity to LDL but also potential errors related to calibration. Although LDL-C (H) showed strong linear correlations with LDL-C (BQ) in the scatter plots, the slopes of their regression lines varied among reagents (Table S8). The slope of Reagent-J especially deviated from those of the others (Fig. 2). Reagent-J uses calibrators whose LDL-C values were determined with frozen reference material certified by the Reference Material Institute for Clinical Chemistry Standards (ReCCS, Kawasaki, Japan). Since ReCCS determines LDL-C levels of this material by the BQ method, matrix effects of frozen serum probably caused considerable errors in LDL-C levels of Reagent-J's calibrators. The other manufacturers set the LDL-C levels of their calibrators using the BQ method with fresh serum. Since calibrators' LDL-C levels are

Table 1
Percentage of serum samples that met the NCEP total error requirement for a single LDL-C determination.

Subjects/TG range	Reagent											
	A	B	C	D	E	F	G	H	I	J	K	L
Non-diseased group (n = 49)	100.0	98.0	100.0	65.3	93.9	98.0	95.9	93.9	95.9	93.9	100.0	98.0
Diseased group												
TG < 11.29 mmol/L (n = 124)	95.2	78.2	81.4	75.0	79.8	86.3	98.4	87.9	87.9	74.2	90.3	91.9
TG < 6.78 mmol/L (n = 115)	97.4	79.1	84.3	76.5	83.2	88.7	92.2	89.6	90.4	73.9	93.0	93.0
TG < 4.52 mmol/L (n = 107)	99.1	84.1	87.9	79.4	86.0	93.5	100.0	92.5	92.5	74.8	97.2	94.3
TG < 11.29 mmol/L w/o Type 1 & 3 dyslipidemia (n = 121)	96.7	79.3	83.5	76.3	80.1	87.6	91.7	89.3	89.3	75.2	92.6	93.3

TG, triglyceride.
A, Denka Seiken; B, Wako; C, Sysmex; D, Serotec; E, Fureiya; F, Kyowa Medex; G, Toyobo; H, Shino-Test; I, Sekisui Medical; J, Ortho Clinical Diagnostics; K, Siemens Healthcare Diagnostics; L, Beckman Coulter.

Table 2
Error component analysis.

Subjects/TG range	Reagent											
	A	B	C	D	E	F	G	H	I	J	K	L
Non-diseased group ($n = 49$)												
CV_b (%)	0.7	0.9	0.8	1.0	1.1	0.5	1.8	0.9	0.8	1.3	0.8	1.2
CV_e (%)	0.5	0.6	0.5	0.6	0.5	0.4	1.1	0.6	0.5	1.1	0.6	0.9
CV_d (%)	4.5	3.8	5.1	5.9	4.1	3.2	7.0	4.9	5.1	6.5	4.9	5.6
CV_t (%)	4.6	4.0	5.2	6.0	4.3	3.3	7.4	5.0	5.1	6.7	5.0	5.8
Mean bias (%)	0.0	0.5	−0.3	1.8	0.7	0.6	−0.1	0.4	0.4	−0.5	−0.1	−0.3
TE (%), for greater of positive or negative limit	9.6	8.8	10.6	14.9	9.7	7.4	14.7	11.0	11.3	13.9	10.3	12.0
Diseased group												
TG < 11.29 mmol/L ($n = 124$)												
CV_e (%)	0.6	0.5	0.6	0.7	0.7	0.5	0.9	0.6	0.6	1.5	0.7	0.8
CV_d (%)	4.7	5.3	9.5	10.0	7.0	5.8	5.9	4.8	4.8	8.3	5.8	5.3
CV_t (%)	4.8	5.4	9.6	10.1	7.1	5.8	6.2	4.9	4.9	8.5	5.9	5.5
Mean bias (%)	0.7	1.4	−0.7	1.6	1.2	1.3	−0.6	1.0	1.1	0.1	0.8	0.1
TE (%), for greater of positive or negative limit	10.8	13.0	20.3	24.3	16.7	13.8	12.2	11.5	11.5	18.7	13.4	11.7
TG < 6.78 mmol/L ($n = 115$)												
CV_e (%)	0.6	0.5	0.6	0.7	0.6	0.5	0.9	0.6	0.6	1.5	0.7	0.8
CV_d (%)	4.3	4.2	7.0	7.7	6.2	5.3	4.7	4.6	4.6	8.4	5.2	5.1
CV_t (%)	4.4	4.3	7.1	7.8	6.3	5.3	5.1	4.7	4.7	8.6	5.3	5.3
Mean bias (%)	0.6	1.4	−0.7	1.5	1.0	1.2	−0.6	1.0	1.0	0.2	0.6	0.1
TE (%), for greater of positive or negative limit	9.8	10.5	14.5	18.9	14.6	12.6	10.0	10.9	11.1	18.9	11.9	11.3
TG < 4.52 mmol/L ($n = 107$)												
CV_e (%)	0.5	0.5	0.6	0.6	0.6	0.5	0.9	0.6	0.6	1.6	0.7	0.8
CV_d (%)	4.1	3.9	5.7	7.8	4.7	3.9	4.5	4.6	4.6	8.6	5.0	5.0
CV_t (%)	4.2	4.1	5.8	7.9	4.9	4.0	4.9	4.7	4.7	8.9	5.1	5.2
Mean bias (%)	0.5	1.2	−0.8	1.4	0.8	1.0	−0.6	0.9	1.0	0.2	0.5	−0.0
TE (%), for greater of positive or negative limit	9.2	9.8	−11.7	18.8	11.1	9.3	−9.9	10.8	10.9	19.6	11.2	1.0
TG < 11.29 mmol/L, w/o Type 1 & 3 dyslipidemia ($n = 121$)												
CV_e (%)	0.6	0.5	0.6	0.7	0.7	0.5	0.9	0.6	0.6	1.5	0.7	0.8
CV_d (%)	4.3	5.3	9.1	9.8	6.8	5.4	5.7	4.6	4.4	8.3	5.4	5.4
CV_t (%)	4.5	5.4	9.2	10.0	6.9	5.5	6.0	4.7	4.6	8.5	5.5	5.5
Mean bias (%)	0.6	1.3	−0.8	1.4	1.0	1.1	−0.6	1.0	0.9	−0.1	0.6	−0.5
TE (%), for greater of positive or negative limit	9.9	12.9	19.2	23.7	15.9	12.8	12.2	10.8	10.6	18.6	12.3	11.6

TE, total error.
We assumed that the sources of error were mainly derived from CV_b (inter-assay), CV_e (intra-assay) and CV_d (subject sample-specific effects). CV_t reflects combined random effects of CV_b, CV_e and CV_d (See Table S4 for details).
We used all the data for this analysis except for 1 outlier from non-diseased group for Toyobo's reagent (Figs. 2 and 3, marked with dotted lines).

relatively low and standard curves are drawn by single point calibration, a small difference in calibration is amplified in the determined LDL-C levels especially in the high LDL-C range. Ideally, manufacturers should set LDL-C levels of their calibrators using fresh serum and the BQ method.

In non-diseased and diseased subjects, our inter- and intra-assay CVs were better than those in Miller's study [12]. Probably, some pre-analytical factors may have caused this discrepancy. For example, we kept all samples below 4 °C and transported them within 24 h. The interval between blood collection and LDL-C determination was shorter in this study than in Miller's study (within 48 h). In both studies, triplicate LDL-C determinations were performed over 3 cycles (See "Methods" section). However, we found neither carryover effects nor sample condensation during triplicate determinations (Tables S2 and S3). Considering the difference in the patient population of both studies, we can say that homogeneous assays have satisfactory precision at least in subjects with common disease and healthy subjects.

Our study has some limitations. First, LDL-C (BQ) contains some inevitable intrinsic errors. The BQ method requires several manual procedures highly dependent on technical skills [9,16]. In some steps, such as transfer of samples to different tubes and cholesterol extraction, 100% recovery is difficult. The inaccuracy of homogeneous assays is overestimated in samples with low LDL-C levels. Second, error component analysis is based on the hypothesis that the mean successive difference between LDL-C (H) and LDL-C (BQ) is very small and continuous. However, it is hard for clinical studies to fit this condition. In fact, there were only a few subjects in the very low [12] or very high LDL-C ranges. Especially in those with high TG range (>4.52 mmol/L), our samples failed to meet this hypothesis. Therefore, patient-specific errors (CV_d) tend to be biased towards higher levels in this analysis. It is safe not to use CV_d for absolute evaluations of certain reagents. In several homogenous assays such as Reagent B, TE values were close to 12% (Table 2). These reagents might have fulfilled the NCTEP TE goal if we had had more samples with low LDL-C. For precise assessment of TE, we need further studies with more patients with very low and very high LDL-C concentrations.

In summary, LDL-C (H) levels are in good agreement with LDL-C (BQ) levels for non-diseased subjects, except for with Reagent-D and Reagent-J. However, performances of 4 reagents (Reagent-C, Reagent-D, Reagent-E, and Reagent-F) are not satisfactory for diseased subjects, especially those with hypertriglyceridemia. Differences in the calibration protocol remain an issue for the standardization of LDL-C (H).

Conflict of interest

Investigators of LDL-C Study Group received no remuneration for conducting this study.

Acknowledgments

The authors would like to thank the 12 Japanese companies (manufacturers and distributors), mentioned in the Methods section, who provided financial support, reagents, calibrators, and controls for this evaluation. We appreciate Ms. Yukari Ichikawa,

Miyuki Ono, Keiko Fujino and Taeko Hotta for their excellent technical assistance.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2012.08.022.

References

[1] National Cholesterol Education Program. Second report of the expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult treatment panel II). Circulation 1994;89:1333–45.
[2] Sullivan DR. Screening for cardiovascular disease with cholesterol. Clin Chim Acta 2002;315:49–60.
[3] Turner RC, Millns H, Neil HA, et al. Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). BMJ 1998;316:823–8.
[4] Baigent C, Blackwell L, Emberson J, , et alFor Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet 2010;376:1670–81.
[5] Willey JZ, Xu Q, Boden-Albala B, et al. Lipid profile components and risk of ischemic stroke: the Northern Manhattan Study (NOMAS). Arch Neurol 2009; 66:1400–6.
[6] Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001;285:2486–97.
[7] Graham I, Atar D, Borch-Johnsen K, , et alFor European Society of Cardiology (ESC) Committee for Practice Guidelines (CPG). European guidelines on cardiovascular disease prevention in clinical practice: executive summary Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Eur Heart J 2007; 28:2375–414.
[8] Teramoto T, Sasaki J, Ueshima H, et al. Executive summary of Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese. J Atheroscler Thromb 2007;14:45–50.
[9] Lipid Research Clinics Program, Manual of Laboratory Operations. 1974 (revised 1982). DHEW (NIH) Publication No 75–628.
[10] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[11] Nakamura M, Taniguchi Y, Yamamoto M, Hino K, Manabe M. Homogenous assay of serum LDL-cholesterol on an automatic analyzer [Abstract]. Clin Chem 1997;43:S260.
[12] Miller WG, Myers GL, Sakurabayashi I, et al. Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. Clin Chem 2010;56:977–86.
[13] Tarugi P, Averna M, Di Leo E, et al. Molecular diagnosis of hypobetalipoproteinemia: an ENID review. Atherosclerosis 2007;195:e19–27.
[14] Foley KF, Silveira MG, Hornseth JM, Lindor KD, McConnell JP. A patient with primary biliary cirrhosis and elevated LDL cholesterol. Clin Chem 2009;55: 187–91.
[15] Centers for Disease Control and Prevention. Manufacturer certification program. http://www.cdc.gov/labstandards/crmln_manufacturers.html [Accessed December 2011].
[16] Abell LL, Levy BB, Brodie RB, Kendall FE. Simplified method for the estimation of total cholesterol in serum, and demonstration of its specificity. J Biol Chem 1952;195:357–66.
[17] van Deventer HE, Miller WG, Myers GL, et al. Non-HDL cholesterol shows improved accuracy for cardiovascular risk score classification compared to direct or calculated LDL cholesterol in a dyslipidemic population. Clin Chem 2011;57:490–501.
[18] Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;327:307–10.
[19] Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. Lancet 1995;346:1085–7.
[20] Nakamura M, Kayamori Y, Sato S, Shimamoto T. Lipid standardization results of Japanese manufacturers by US Cholesterol Reference Method Laboratory Network certification protocols and reagents' specificity and performance. In: Kramer MA, editor. Focus on cholesterol research. 1st ed. Hauppauge: Nova Science Publishers; 2006. p. 75–146.
[21] Usui S, Kakuuchi H, Okamoto M, Mizukami Y, Okazaki M. Differential reactivity of two homogeneous LDL-cholesterol methods to LDL and VLDL subfractions, as demonstrated by ultracentrifugation and HPLC. Clin Chem 2002; 48:1946–54.
[22] Yamada K, Tsuji N, Fujita T, et al. Comparison of four direct homogeneous methods for the measurement of low-density lipoprotein cholesterol. Clin Lab 2010;56:327–33.
[23] Sakaue T, Hirano T, Yoshino G, Sakai K, Takeuchi H, Adachi M. Reactions of direct LDL-cholesterol assays with pure LDL fraction and IDL: comparison of three homogeneous methods. Clin Chim Acta 2000;295:97–106.
[24] Yamashita S, Nakamura M, Koizumi H, et al. Evaluation of a homogeneous assay for measuring LDL-cholesterol in hyperlipidemic serum specimens. J Atheroscler Thromb 2008;15:82–6.
[25] Yamashita S, Kawase R, Nakaoka H, et al. Differential reactivities of four homogeneous assays for LDL-cholesterol in serum to intermediate-density lipoproteins and small dense LDL: comparisons with the Friedewald equation. Clin Chim Acta 2009;410:31–8.
[26] Kosuge K, Miida T, Takahashi A, et al. Estimating the fasting triglyceride concentration from the postprandial HDLcholesterol and apolipoprotein CIII concentrations. Atherosclerosis 2006;184:413–9.
[27] Tanno K, Okamura T, Ohsawa M, et al. Comparison of low-density lipoprotein cholesterol concentrations measured by a direct homogeneous assay and by the Friedewald formula in a large community population. Clin Chim Acta 2010;411:1774–80.
[28] Fukushima Y, Hirayama S, Ueno T, et al. Small dense LDL cholesterol is a robust therapeutic marker of statin treatment in patients with acute coronary syndrome and metabolic syndrome. Clin Chim Acta 2011;412:1423–7.
[29] Di Angelantonio E, Sarwar N, Perry P, , et alFor the Emerging Risk Factors Collaboration. Major lipids, apolipoproteins, and risk of vascular disease. JAMA 2009;302:1993–2000.
[30] Mora S, Rifai N, Buring JE, Ridker PM. Comparison of LDL cholesterol concentrations by Friedewald calculation and direct measurement in relation to cardiovascular events in 27 331 women. Clin Chem 2009;55:888–94.

Original Article

Does High-Sensitivity C-Reactive Protein or Low-Density Lipoprotein Cholesterol Show a Stronger Relationship with the Cardio-Ankle Vascular Index in Healthy Community Dwellers?: the KOBE Study

Aya Higashiyama[1,2], Ichiro Wakabayashi[1], Yoshimi Kubota[2], Yoshiko Adachi[1], Akiko Hayashibe[1], Kunihiro Nishimura[2,3], Daisuke Sugiyama[2,4], Aya Kadota[2,5], Hironori Imano[2,6], Naomi Miyamatsu[2,7], Yoshihiro Miyamoto[2,3] and Tomonori Okamura[2,4]

[1]Department of Environmental and Preventive Medicine, Hyogo College of Medicine, Hyogo, Japan
[2]Foundation for Biomedical Research and Innovation, Hyogo, Japan
[3]Department of Preventive Medicine and Epidemiologic Informatics, National Cerebral and Cardiovascular Center, Osaka, Japan
[4]Department of Preventive Medicine and Public Health, School of Medicine, Keio University, Tokyo, Japan
[5]Department of Health Science, Shiga University of Medical Science, Shiga, Japan
[6]Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan
[7]Department of Clinical Nursing, Shiga University of Medical Science, Shiga, Japan

Aim: High-sensitivity C-reactive protein (hs-CRP) identifies individuals at risk for cardiovascular disease (CVD) without an increased level of low-density lipoprotein cholesterol (LDL-C). The present study was performed to compare hs-CRP and LDL-C in association with the cardio-ankle vascular index (CAVI) in Japanese community dwellers considered to be at low risk for atherosclerosis from their level of traditional CVD risk factors.

Methods: A community-based study involving 386 healthy Japanese (261 men and 125 women) without a history of CVD and medications for hypertension, diabetes, and dyslipidemia was performed. Multiple adjustments were performed with linear regression models to estimate the association between CAVI and hs-CRP or LDL-C levels. The participants were divided into four groups on the basis of whether they were above or below the median hs-CRP and LDL-C values, and CAVI was compared among the four groups by analysis of covariance after adjusting for confounders.

Results: In multiple linear regression models, hs-CRP showed a significant positive association with CAVI; however, no clear association was observed between CAVI and LDL-C. These results were similar in the analyses among the participants with LDL-C <140 mg/dL or hs-CRP <1.0 mg/L. CAVI was higher in the groups with high hs-CRP than in those with low hs-CRP, irrespective of LDL-C; however, CAVI was highest in the group with high LDL-C and high hs-CRP.

Conclusions: The present study suggests that hs-CRP could be a better risk factor assessor for atherosclerosis than LDL-C in individuals considered to be at low risk for atherosclerosis assessed by their traditional CVD risk factors.

J Atheroscler Thromb, 2012; 19:1027-1034.

Key words; Community-based study, Atherosclerosis, Inflammation, Epidemiology

Address for correspondence: Aya Higashiyama, Department of Environmental and Preventive Medicine, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan
E-mail: ahigashi@hyo-med.ac.jp
Received: February, 23, 2012
Accepted for publication: May 15, 2012

Introduction

Both low-density lipoprotein cholesterol (LDL-C) and high-sensitivity C-reactive protein (hs-CRP) are associated with an increased risk for cardiovascular disease (CVD)[1-4]; however, in the Women's Health Study, almost half of the CVD events occurred in par-

ticipants with LDL-C <130 mg/dL, and hs-CRP identified at-risk individuals with low LDL-C levels[5]. Furthermore, a large-scale, randomized, clinical trial (JUPITER) reported that lipid-lowering therapy reduced incident CVD in individuals with elevated hs-CRP who did not meet the criteria for lipid-lowering drug therapy[6]. Thus, hs-CRP could be a useful adjuvant guide for therapy to complement established traditional risk factors such as dyslipidemia[7].

Compared to the Western population, the Japanese have been reported to have lower cholesterol levels[8]. Moreover, CRP has been reported to be much lower in the general population without a history of CVD in Japan than in Western countries[9, 10]. Thus, whether such a low CRP level is still associated with atherosclerosis in the Japanese general population with low LDL-C should be investigated.

The cardio-ankle vascular index (CAVI) is a novel arterial-stiffness parameter[11, 12]. The CAVI has been demonstrated to be associated with CVD[13-15], and it is considered to be a good marker of atherosclerosis[16].

Thus, a cross-sectional study, involving 386 Japanese individuals without a past history of CVD and who were free from medication for hypertension, diabetes, and dyslipidemia, was conducted to investigate the relationships among LDL-C, hs-CRP, and CAVI in a general population that was considered to be at low risk for CVD.

Aim

The aim of the present study was to compare hs-CRP and LDL-C levels in association with the CAVI in Japanese community dwellers considered to be at low risk for atherosclerosis from their level of traditional cardiovascular (CVD) risk factors.

Methods

Study Participants

Data from the baseline survey in the Kobe Orthopedic and Biomedical Epidemiological study (KOBE study) were analyzed. The KOBE study is a population-based cohort study in which the endpoint is considered worsening of quality of life or CVD risk factors, such as hypertension, diabetes mellitus, and dyslipidemia. Study participants were volunteers aged 40 to 74 years who were residents of Kobe city, one of the major urban areas in Japan. The participants had to meet the following criteria: 1) not currently on medications for hypertension, dyslipidemia, and diabetes mellitus; and 2) no past history of CVD and cancer. The participants were recruited by Kobe municipal government, such as on websites and in magazines, or by advertising in newspapers and by posters or leaflets in public facilities, universities, and companies in Kobe city. Written informed consent was obtained from each participant. As part of the baseline survey of this cohort study, CAVI was performed in 403 individuals from July 2010 to October 2011. Of these, 17 participants were excluded because of the following reasons: did not meet the study criteria ($n=12$); triglyceride ≥400 mg/dL ($n=1$); and hs-CRP >10.0 mg/L ($n=4$). The remaining 386 individuals (261 men and 125 women) were included in the present study.

The present study was approved by the Ethics Committee of the Institute of Biomedical Research and Innovation.

Data Collection and Standardization

Height and weight were measured while wearing socks and light clothing, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). The participants were asked to respond to questionnaires about lifestyle-related factors, such as medication, smoking (current smoker or not), and alcohol consumption (current drinker or not).

Fasting blood samples (after fasting for at least 10 h) were obtained from all participants, and blood samples were tested by one commissioned clinical laboratory center (SRL Inc., Tokyo, Japan). Plasma glucose, serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride levels were measured by enzymatic methods. Then, LDL-C was calculated by Friedewald's formula. Serum hs-CRP was measured using a BN II nephelometer (Dade Behring, Deerfield, IL, USA).

CAVI was measured using a VaSera CAVI instrument (Fukuda Denshi Co. Ltd., Tokyo, Japan). Briefly, cuffs were applied to the bilateral upper arms and ankles with the participant lying in the supine position. After a 5-min rest, the examination was performed. CAVI was calculated by the following formula:

$$CAVI = a\{(2\rho/\Delta P) \times \ln(Ps/Pd)\, PWV^2\} + b$$

where Ps is systolic blood pressure, Pd is diastolic blood pressure, PWV is pulse wave velocity, ΔP is Ps − Pd, ρ is blood density, and a and b are constants. Scale conversion was performed to compare CAVI with PWV (Hasegawa's method). The VaSera is equipped with both measurement and calculation systems and automatically calculated CAVI.

Table 1. Characteristics of the participants

	Men	Women
Number of participants	261	125
Age (years)	61 ± 9	63 ± 8
BMI (kg/m^2)	22.8 ± 2.7	20.9 ± 2.4
Systolic blood pressure (mmHg)	128 ± 15	126 ± 16
Diastolc blood pressure (mmHg)	84 ± 9	79 ± 10
Heart rate (beat/min)	57 ± 9	60 ± 8
Serum total cholesterol (mg/dL)	205 ± 29	231 ± 31
LDL-cholesterol (mg/dL)	124 ± 28	140 ± 28
Fasting glucose (mg/dL)	93 ± 13	88 ± 6
HbA1c (%)	5.2 ± 0.5	5.3 ± 0.3
Current smoking (%)	10.7	1.6
Current alcohol drinking (%)	76.2	32.8
Hs-CRP (mg/L)	0.27 (0.05-4.03)	0.25 (0.05-5.38)
CAVI	8.1 ± 0.9	8.0 ± 1.1

BMI: body mass index; LDL-cholesterol: low-density lipoprotein cholesterol; hs-CRP: high sensitivity C-reactive protein. Values are the mean ± SD unless sepcified otherwise. Values of smoking and alcohol drinking are percentages. Values of hs-CRP are the median (range).

Statistical Analysis

Sex-specific and sex-combined analyses were performed. Multiple adjustments were performed with linear regression models to estimate the association between CAVI and hs-CRP (log-transformed) or LDL-C level. Model 1 included sex, age, LDL-C, and hs-CRP, and Model 2 included the variables in Model 1 plus systolic blood pressure, heart rate, BMI, HDL-C, and fasting glucose. The adjusted coefficient of determination (adjusted R^2) was also calculated.

Furthermore, the participants were divided into four groups classified by the combination of the medians for LDL-C and hs-CRP. Then, CAVI was compared among the four groups by analysis of covariance (ANCOVA) after adjustment for age, sex, systolic blood pressure, heart rate, HDL-C, fasting glucose, BMI, smoking, and alcohol drinking to investigate the relationships between CAVI and the combinations of LDL-C and hs-CRP.

All p values were two-tailed and the significance level was set at $p<0.05$. The statistical package SPSS 15.0J for Windows (SPSS, Tokyo, Japan) was used to perform these analyses.

Results

The mean age of the participants was 62 ± 9 years, the mean LDL-C was 129 ± 29 mg/dL, and the median hs-CRP was 0.26 (0.05-5.38) mg/L. The geometric mean hs-CRP was 0.28 mg/L. The mean CAVI was 8.1 ± 1.0.

Table 1 shows the sex-specific characteristics of the participants. The mean age was 61 ± 9 years in men and 63 ± 8 years in women. The mean LDL-C in women was higher than the normal limit, but they did not meet the criteria for lipid-lowering drug therapy[17]. The mean blood pressure and glucose level did not meet the criteria for hypertension or diabetes mellitus[18, 19].

The associations between CAVI and hs-CRP (log-transformed) or LDL-C in multiple linear regression models are presented in **Table 2** (sex-specific) and **Table 3** (sex-combined). In all models, hs-CRP showed a significant positive association with CAVI. In women, the standardized coefficient of hs-CRP was the second largest next to age in Model 2. On the other hand, no clear association was observed between CAVI and LDL-C. Adding uric acid, smoking (current or non-current), and alcohol drinking (current or non-current) as independent variables, or substituting waist circumference for BMI in Model 2 did not change these results (data not shown). In sex-combined analyses, the same analyses were performed in the participants with LDL-C < 140 mg/dL (n=253) and in those with hs-CRP < 1.0 mg/L (n=347); the results were similar (data not shown).

Table 4 shows the associations between CAVI and the four groups classified by combinations of LDL-C and hs-CRP. CAVI was higher in the groups with high hs-CRP than in those with low hs-CRP, irrespective of LDL-C levels. CAVI was highest in the participants with high hs-CRP and high LDL-C, and

Table 2. Association among CAVI, LDL-C and hs-CRP (log-transformed) in the multiple linear regression models by sex

Dependent variables	Men				Women			
	Independent variables: CAVI				Independent variables: CAVI			
	Coefficient	95%CI	Standardized coefficient	*p* value	Coefficient	95%CI	Standardized coefficient	*p* value
Model 1								
Age (years)	0.056	0.046-0.066	0.566	<0.001	0.072	0.053-0.092	0.542	<0.001
LDL-C (mg/dL)	0.001	-0.003-0.004	0.020	0.692	0.002	-0.004-0.007	0.044	0.553
Ln hs-CRP (mg/L)	0.111	0.020-0.202	0.122	0.017	0.181	0.036-0.326	0.183	0.015
	R^2=0.353				R^2=0.371			
Model 2								
Age (years)	0.047	0.037-0.057	0.473	<0.001	0.068	0.047-0.088	0.507	<0.001
BMI (kg/m^2)	-0.057	-0.093--0.021	-0.169	0.002	-0.047	-0.116-0.022	-0.107	0.176
Systolic blood pressure (mmHg)	0.011	0.005-0.018	0.186	0.001	0.011	0.000-0.021	0.158	0.049
Heart rate (beat/min)	0.006	-0.004-0.016	0.063	0.215	0.003	-0.017-0.023	0.024	0.756
Fasting glucose (mg/dL)	0.008	0.001-0.015	0.115	0.022	-0.022	-0.047-0.004	-0.126	0.103
HDL-C (mg/dL)	-0.005	-0.012-0.001	-0.084	0.115	-0.004	-0.015-0.006	-0.062	0.407
LDL-C (mg/dL)	0.001	-0.002-0.004	0.029	0.550	0.002	-0.004-0.007	0.041	0.590
Ln hs-CRP (mg/L)	0.103	0.012-0.195	0.114	0.027	0.225	0.072-0.378	0.227	0.004
	R^2=0.414				R^2=0.392			

BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CAVI: cardio ankle vascular index. 95%CI: 95% confidence interval. ln hs-CRP: log-transformed high sensitivity C-reactive protein.
R^2: adjusted coefficient of determination.
Multiple adjustments were performed with linear regression models: Model 1 included age, LDL-C, and hs-CRP (log-transformed). Model 2 included variables in model 1 plus systolic blood pressure, heart rate, BMI, HDL-C, and fasting glucose.

lowest in those with low hs-CRP and low LDL-C. In sex-combined analyses, the relationships between the four groups and multivariate-adjusted CAVI were significant. The results were similar when the same analyses were performed in sex-combined participants with CRP < 1.0 mg/L (n=347) (data not shown).

Discussion

In the present study, hs-CRP showed a significant positive association with CAVI; on the other hand, no clear association between LDL-C and CAVI was observed. However, when CAVI was compared among the four groups divided by the combination of LDL-C and hs-CRP, the participants with high-LDL and high hs-CRP showed the highest CAVI among the four groups.

Both LDL-C and hs-CRP are associated with an increased risk for CVD[1-4]; however, in the Women's Health Study, almost half of the CVD events occurred in participants with LDL-C <130 mg/dL, and hs-CRP identified at-risk individuals with low LDL-C levels[5]. A large-scale, randomized, clinical trial (JUPITER) reported that lipid-lowering therapy reduced incident CVD in individuals with elevated hs-CRP levels who did not meet the criteria for lipid-lowering drug therapy[6]. From these results, individuals with high LDL-C are naturally considered to be at high risk for atherosclerosis; however, CRP is a candidate adjuvant guide for the risk assessment of future atherosclerosis or therapy in individuals without established traditional risk factors such as dyslipidemia[5-7]. Thus, the present study was performed to investigate whether CRP could identify individuals at high risk for atherosclerosis in comparison with LDL-C in participants who were considered to be at low risk from their traditional CVD risk factors including LDL-C. The participants in the Women's Health Study were naturally considered to be at low risk for CVD because their LDL-C was <130 mg/dL; however, the participants in the present study might be even healthier than those in the previous study. Not only the mean LDL-C level in both sexes combined (129 mg/dL), but also the mean blood pressure and glucose levels in both men and women were considered to be within normal levels in the present study, and BMI and hs-CRP levels were higher in the previous study. In addition, the very low prevalence of smoking was one of

Table 3. Association among CAVI, LDL-C and hs-CRP (log-transformed) in the multiple linear regression models in all participants

Dependent variables	All participants			
	Independent variables: CAVI			
	Coefficient	95%CI	Standardized coefficient	*p* value
Model 1				
Age (years)	0.061	0.052-0.070	0.555	<0.001
Sex (men)	0.196	0.023-0.368	0.095	0.026
LDL-C (mg/dL)	0.001	−0.002-0.004	0.037	0.392
Ln hs-CRP (mg/L)	0.138	0.061-0.216	0.148	<0.001
		R^2=0.358		
Model 2				
Age (years)	0.053	0.043-0.062	0.481	<0.001
Sex (men)	0.194	−0.002-0.389	0.094	0.052
BMI (kg/m^2)	−0.052	−0.084-−0.020	−0.148	0.002
Systolic blood pressure (mmHg)	0.011	0.005-0.016	0.168	<0.001
Heart rate (beat/min)	0.005	−0.004-0.014	0.050	0.239
Fasting glucose (mg/dL)	0.005	−0.002-0.012	0.058	0.172
HDL-C (mg/dL)	−0.004	−0.010-0.001	−0.074	0.111
LDL-C (mg/dL)	0.001	−0.001-0.004	0.044	0.299
Ln hs-CRP (mg/L)	0.144	0.065-0.223	0.153	<0.001
		R^2=0.399		

BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CAVI: cardio ankle vascular index. 95%CI: 95% confidence interval. ln hs-CRP: log-transformed high sensitivity C-reactive protein.
R^2: adjusted coefficient of determination.
Multiple- adjustments were performed with the linear regression models: Model 1 included sex, age, LDL-C, and hs-CRP (log-transformed). Model 2 included variables in model 1 plus systolic blood pressure, heart rate, BMI, HDL-C, and fasting glucose.

the characteristics of the present study (men 10.7%, women 1.6%). Although the present study was cross-sectional in design and the independent variable was CAVI and not CVD events, the hs-CRP showed a stronger association with CAVI than LDL-C.

The strength of the present study was that the participants were a general population without a history of CVD and on no medication for hypertension, diabetes, and dyslipidemia. Because healthy community dwellers seldom visit hospitals to undergo examinations for atherosclerosis, it is usually difficult to collect data about the parameters for atherosclerosis in relatively healthy individuals compared to patients. Occupational health data could be a candidate to investigate the present study question, but it might be difficult to assess the participants' atherosclerosis condition because of the healthy worker effect[20] or younger age compared to the general population.

One of the problems of using hs-CRP as a risk factor for atherosclerosis might be that hs-CRP could increase in various conditions other than atherosclerosis. The American Heart Association and the Centers for Disease Control (AHA/CDC) position paper suggests that CRP >10.0 mg/L might indicate the need to consider an ongoing infectious or inflammatory disease[21]. Accordingly, the data were analyzed after the exclusion of participants with CRP ≥10 mg/L. In addition, the same analyses were performed in participants with CRP <1.0 mg/L, who were considered to be in a low-risk category for coronary artery disease according to the recommendation by the AHA/CDC[21]. In these analyses, the results were similar to those in overall participants. Thus, low hs-CRP might be associated with atherosclerosis, and hs-CRP screening could be useful to predict future CVD events from the early stage of atherosclerosis, especially in individuals without traditional CVD risk factors.

For LDL-C, a previous study also showed no clear association between LDL-C and CAVI in a relatively healthy general rural population without a history of CVD[14]; however, Miyashita *et al.* reported that ezetimibe improved CAVI in addition to lowering LDL-C in type 2 diabetic patients, and the patients with a high response to ezetimibe followed by achievement of the goal of LDL-C <120 mg/dL showed significant improvement of CAVI[22]. They concluded

Table 4. Combination of LDL-C and hs-CRP (below the median or the median and above) and CAVI

	Combination of LDL-C and hs-CRP				*p* value
	LDL-C < median hs-CRP < median	LDL-C ≥ median hs-CRP < median	LDL-C < median hs-CRP ≥ median	LDL-C ≥ median hs-CRP ≥ median	
Men					
Number of participants	75	55	56	75	
CAVI (mean ± SD)	7.9 ± 0.9	8.0 ± 0.8	8.2 ± 0.8	8.2 ± 0.9	0.035
CAVI (multivariate-adjusted mean and 95%CI)*	8.0 (7.8-8.1)	8.0 (7.8-8.2)	8.1 (8.0-8.3)	8.2 (8.0-8.4)	0.215
Women					
Number of participants	35	27	27	36	
CAVI (mean ± SD)	7.7 ± 0.8	7.7 ± 1.0	8.1 ± 1.1	8.4 ± 1.3	0.019
CAVI (multivariate-adjusted mean and 95%CI)*	7.8 (7.5-8.0)	7.8 (7.5-8.1)	8.1 (7.8-8.5)	8.3 (8.0-8.6)	0.053
Men and women					
Number of participants (men %)	111 (74.8)	82 (56.1)	80 (82.5)	113 (58.4)	
CAVI (mean ± SD)	7.8 ± 0.9	7.9 ± 0.9	8.1 ± 0.9	8.3 ± 1.1	0.001
CAVI (multivariate-adjusted mean and 95%CI)†	7.9 (7.8-8.1)	7.9 (7.8-8.1)	8.1 (7.9-8.2)	8.3 (8.1-8.4)	0.003

P value: *p* value of analysis of variance or analysis of covariance. SD: standard deviation.
hs-CRP: high sensitivity C-reactive protein. 95%CI: 95% confidence interval. CAVI: cardio-ankle vascular index.
Median of LDL-C; men 122 mg/dL, women 141 mg/dL, men and women 127 mg/dL.
Median of hs-CRP; men 0.27 mg/L, women 0.25 mg/L, men and women 0.26 mg/L.
Multivariate-adjusted mean*: adjusted for age, systolic blood pressure, heart rate, fasting glucose, BMI, HDL-C, smoking (current or non-current), and alcohol drinking (current or non-current).
Multivariate-adjusted mean †: adjusted for sex in addition to variables adjusted in the above model*.

that diabetic patients had shown high cholesterol absorption and that ezetimibe might have the potential to ameliorate vascular stiffness through the inhibition of cholesterol absorption[22]. Furthermore, Miyoshi *et al.* have reported that CAVI was independently associated with LDL-C in multivariate analysis among individuals with significant coronary stenosis, defined as 50% or greater luminal diameter narrowing assessed by coronary angiography[15]. Thus, the relationship between CAVI and LDL-C may be different among high-risk participants, including patients with diabetes or coronary artery disease, and low-risk participants. In addition, in the present study, a tendency toward a positive association between LDL-C and CAVI was observed in women when the relationship between CAVI and the tertile of LDL-C was evaluated, although the relationship was not significant (data not shown). Moreover, CAVI was highest in participants with high hs-CRP and high LDL-C among the four groups in the present study. According to Shirai *et al.*, hyperlipidemia per se does not immediately increase arterial wall stiffness[12]. After the accumulation of cholesterol in the lipid pool, oxidative stress generates oxysterol, which is highly toxic and enhances inflammation, followed by the onset of atherosclerosis; therefore, CAVI may increase under certain conditions in dyslipidemia[12]. Thus, hs-CRP might be a marker of inflammation due to oxidative stress, and an association between hs-CRP and arterial wall stiffness might be observed in the present study. Thus, the association between LDL-C and CAVI including hs-CRP remains a future topic to be investigated, especially in further combination with apolipoprotein B14 or oxidized LDL-C[23].

The present study had several limitations. First, because it was a cross-sectional study, causality could not be determined, and the results should be confirmed by future prospective studies. Second, hs-CRP elevation might have been caused by diseases other than atherosclerosis, of which the participant was unaware. Third, because the participants in the present study were considered to be volunteers with high health consciousness, application of the results in the present study to the general population should be carefully considered. Fourth, because CAVI is still a new device for the assessment of atherosclerosis and

the relationship with future CVD events has not been sufficiently investigated in a prospective study, it is unconfirmed that the relationships among hs-CRP, LDL-C and CAVI reflect those among hs-CRP, LDL-C and future CVD events in a healthy general population.

Conclusions

In the cross-sectional study performed in an urban Japanese general population who were considered to be at low risk for atherosclerosis without medication for hypertension, diabetes, and dyslipidemia, hs-CRP was positively associated with CAVI; however, no clear association between LDL-C and CAVI was observed. Thus, the present study indicates that hs-CRP could be a better risk factor for atherosclerosis than LDL-C in individuals who seem to be at low risk when assessed by established traditional CVD risk factors.

Acknowledgements

The authors would like to express their sincere appreciation to the volunteers involved in the administration of the KOBE study and all of the research staff.

Notice of Grant Support

This study was supported by Grants from the Regional Innovation Cluster Program, Global Type, Ministry of Education, Culture, Sports, Science and Technology, by a Grant-in-Aid for Researchers, Hyogo College of Medicine, 2010, by a Grant-in-Aid for Young Scientists B 23790711 from the Japan Society for the Promotion of Science, by the Intramural Research Fund of the National Cerebral and Cardiovascular Center (22-4-5), and by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (B 21390211, B 23390178, C 23590835).

Conflicts of Interest

There are no conflicts of interest in the present study.

References

1) Pekkanen J, Linn S, Heiss G, Suchindran CM, Leon A, Rifkind BM, Tyroler HA: Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. N Engl J Med,1990; 322: 1700-1707
2) Okamura T, Kokubo Y, Watanabe M, Higashiyama A, Miyamoto Y, Yoshimasa Y, Okayama A: Low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol and the incidence of cardiovascular disease in an urban Japanese cohort study: The Suita study. Atherosclerosis, 2009; 203: 587-592
3) Ridker PM: Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation, 2003; 107: 363-369
4) Iso H, Cui R, Date C, Kikuchi S, Tamakoshi A; JACC Study Group: C-reactive protein levels and risk of mortality from cardiovascular disease in Japanese: the JACC Study. Atherosclerosis, 2009; 207: 291-297
5) Ridker PM, Rifai N, Rose L, Buring JE, Cook NR: Comparison of C-reactive protein and low-density lipoprotein levels in the prediction of first cardiovascular events. N Engl J Med, 2002; 347: 1557-1565
6) Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastellein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ: Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med, 2008; 359: 2195-2207
7) Libby P, Ridker PM: Inflammation and atherosclerosis: Role of C-reactive protein in risk assessment. Am J Med, 2004; 116: 9S-16S
8) Ueshima H: Explanation for Japanese paradox: prevention of increase in coronary heart disease and reduction in stroke. J Atheroscler Thromb, 2007; 14: 278-286
9) El-Saed A, Curb JD, Kadowaki T, Okamura T, Sutton-Tyrrell K, Masaki K, Seto TB, Takamiya T, Choo J, Edmundowicz D, Evans RW, Fujiyoshi A, Nakamura Y, Miura K, Shin C, Kuller LH, Ueshima H, Sekikawa A: The prevalence of aortic calcification in Japanese compared to white and Japanese-American middle-aged men is confounded by the amount of cigarette smoking. Int J Cardiol, 2012, in press
10) Yamada S, Gotoh T, Nakashima Y, Kayaba K, Ishikawa S, Nago N, Nakamura Y, Itoh Y, Kaiji E: Distribution of serum C-reactive protein and its atherosclerotic risk factors in a Japanese population: Jichi Medical School Cohort Study. Am J Epidemiol, 2001; 153: 1183-1190
11) Noike H, Nakamura K, Sugiyama Y, Iizuka T, Shimizu K, Takahashi M, Hirano K, Suzuki M, Mikamo H, Nakagami T, Shirai K: Changes in cardio-ankle vascular index in smoking cessation. J Atheroscler Thromb, 2010; 17: 517-525
12) Shirai K, Hiruta N, Song M, Kurosu T, Suzuki J, Tomaru T, Miyashita Y, Saiki A, Takahashi M, Suzuki K, Takata M: Cardio-ankle vascular index (CAVI) as a novel indicator of arterial stiffness: theory, evidence and perspectives. J Atheroscler Thromb, 2011; 18: 924-938
13) Nakamura K, Tomaru T, Yamamura S, Miyashita Y, Shirai K, Noike H: Cardio-ankle vascular index is a candidate predictor of coronary atherosclerosis. Circ J, 2008; 72: 598-604
14) Izuhara M, Shioji K, Kadota S, Baba O, Takeuchi Y, Uegaito T, Mutsuo S, Matsuda M: Relationship of cardio-

ankle vascular index (CAVI) to carotid and coronary arteriosclerosis. Circ J, 2008; 72: 1762-1767
15) Miyoshi T, Doi M, Hirohata S, Sakane K, Kamikawa S, Kitawaki T, Kaji Y, Kusano KF, Ninomiya Y, Kusachi S: Cardio-ankle vascular index is independently associated with the severity of coronary atherosclerosis and left ventricular function in patients with ischemic heart disease. J Atheroscler Thromb, 2010; 17: 249-258
16) Kadota K, Takamura N, Aoyagi K, Yamasaki H, Usa T, Nakazato M, Maeda T, Wada M, Nakashima K, Abe K, Takeshima F, Ozono Y: Availability of cardio-ankle vascular index (CAVI) as a screening tool for atherosclerosis. Circ J, 2008; 72: 304-308
17) Teramoto T, Sasaki J, Ueshima H, Egusa G, Kinoshita M, Shimamoto K, Daida H, Biro S, Hirobe K, Funahashi T, Yokote K, Yokode M; Japan Atherosclerosis Society (JAS) Committee for Epidemiology and Clinical Management of Atherosclerosis: Diagnostic criteria for dyslipidemia. Executive summary of Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese. J Atheroscler Thromb, 2007; 14: 155-158
18) Ogihara T, Kikuchi K, Matsuoka H, Fujita T, Higaki J, Horiuchi M, Imai Y, Imaizumi T, Ito S, Iwao H, Kario K, Kawano Y, Kim-Mitsuyama S, Kimura G, Matsubara H, Matsuura H, Naruse M, Saito I, Shimada K, Shimamoto K, Suzuki H, Takishita S, Tanahashi N, Tsuchihashi T, Uchiyama M, Ueda S, Ueshima H, Umemura S, Ishimitsu T, Rakugi H; Japanese Society of Hypertension Committee: The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009). Hypertens Res, 2009; 32: 3-107
19) American Diabetes Association. Standards of medical care in diabetes-2010. Diabetes Care, 2010; 33 (suppl 1): S11-S61
20) CY Li, FC Sung: A review of the healthy worker effect in occupational epidemiology. Occup. Med, 1999; 49: 225-229
21) Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F; Centers for Disease Control and Prevention; American Heart Association: Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation, 2003; 107: 499-511
22) Miyashita Y, Endo K, Saiki A, Ban N, Nagumo A, Yamaguchi T, Kawana H, Nagayama D, Ohira M, Oyama T, Shirai K: Effect of Ezetimibe monotherapy on lipid metabolism and arterial stiffness assessed by Cardio-Ankle Vacular Index in type 2 diabetic patients. J Atheroscler Thromb, 2010; 17: 1070-1076
23) Inoue N, Okamura T, Kokubo Y, Fujita Y, Sato Y, Nakanishi M, Yanagida K, Kakino A, Iwamoto S, Watanabe M, Ogura S, Otsui K, Matsuda H, Uchida K, Yoshimoto R, Sawamura T: LOX index, a novel predictive biochemical marker for coronary heart disease and stroke. Clin Chem, 2010; 56: 550-558

平成 24 年度厚生労働科学研究費補助金
循環器疾患・糖尿病等生活習慣病対策総合研究事業
「慢性期ハイリスク者、脳卒中および心疾患患者に適切な早期受診を
促すための地域啓発研究」

平成 24 年度　総括・分担研究報告書

発　　行　　平成 25 年 3 月
発 行 者　　「慢性期ハイリスク者、心疾患患者に適切な早期受診を促すための
地域啓発研究」班

研究代表者　　宮本　恵宏
〒565-8565
大阪府吹田市藤白台 5-7-1
独立行政法人国立循環器病研究センター予防健診部
TEL:06-6833-5012　FAX:06-6833-5300

印　　刷　　田中耕印刷株式会社
〒538-0054
大阪市鶴見区緑 3-13-5
TEL:06-6912-8400　FAX:06-6912-6572