Cholesterol Levels of Six Fractionated Serum Lipoproteins and its Relevance to Coronary Heart Disease Risk Scores

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**Aim:** Evaluation of serum lipoprotein profiles including triglyceride (TG)-rich lipoprotein, that is, intermediate-density lipoprotein (IDL), very low-density lipoprotein (VLDL), and chylomicron (CM) remnant is important to manage coronary heart disease (CHD) risk. The purpose of this study was to investigate CHD or cardiovascular disease (CVD) risk scores with cholesterol levels of six fractionated lipoprotein classes {high-density lipoprotein [HDL], low-density lipoprotein [LDL], IDL, VLDL, CM including CM remnant, and lipoprotein (a) [Lp (a)]} in Japanese healthy men.

**Methods:** The present study enrolled 161 healthy men without any medications. Lipoprotein profiles (fractionated lipoprotein cholesterol levels) were measured by anion-exchange high-performance liquid chromatography (AEX-HPLC) method and were compared with age, estimated glomerular filtration rate (eGFR), and three risk scores, that is, NIPPON DATA, Hisayama risk predicting model, and Suita score.

**Results:** Levels of LDL-cholesterol (C), VLDL-C, and CM-C significantly differed with age, while values of HDL-C, IDL-C, and Lp(a)-C were not different. The eGFR inversely correlated with LDL-C, IDL-C, VLDL-C, and CM-C. In a stepwise multiple logistic regression analysis, VLDL-C only correlated independently with eGFR. Three risk scores significantly correlated with CM-C.

**Conclusions:** These results suggested that VLDL-C concentration contributes to an increased risk at early stages of renal dysfunction, and CM-C may serve as a marker for estimating CHD risk in Japanese healthy men.

Key words: Anion-exchange high-performance liquid chromatography, Triglyceride-rich lipoprotein, Estimated glomerular filtration rate, Coronary heart disease

**Introduction**

Increased low-density lipoprotein-cholesterol (LDL-C) and decreased high-density lipoprotein-cholesterol (HDL-C) are primary risk factors for coronary heart disease (CHD)². LDL-C is regarded as a primary target for the treatment of dyslipidemia because LDL-C is strongly associated with CHD risk³.⁴. Previous articles reported the association of lipoproteins other than LDL with CHD risk. Very low-density lipoprotein cholesterol (VLDL-C) was shown to be a significant predictor of CHD events in Framingham Heart Study⁵. Intermediate-density lipoprotein-cholesterol (IDL-C) was reported to have an association with the severity of CHD⁶. Furthermore, IDL-C is significantly increased in type III hyperlipidemia⁷. Individuals with chronic kidney disease (CKD) are known to have high levels of triglyceride (TG)-rich lipoproteins including IDL and VLDL⁸. Raised IDL-C was associated with aortic sclerosis in hemodialysis patients⁹.

We have previously established an analysis method for determining cholesterol concentrations of six lipoprotein classes {HDL, LDL, IDL, VLDL, chylomicron [CM] including remnant, and lipoprotein (a) [Lp (a)]} by anion-exchange high-performance liquid chromatography (AEX-HPLC) method¹⁰.¹¹. Cholesterol levels of HDL, LDL, IDL, VLDL, and CM measured by
AEX-HPLC were sufficiently correlated with those measured by an ultracentrifugation method\(^{10}\), and Lp (a) cholesterol (Lp (a)-C) measured by AEX-HPLC was correlated with Lp (a) mass using an immunoturbidimetric reagent\(^{11}\).

Previous studies showed the relationship between lipoprotein cholesterol levels and age. The Framingham study showed changes in mean and percentiles of HDL-C, LDL-C, and VLDL-C by age\(^{12}\). Arai et al. reported changes in total cholesterol (TC), HDL-C, LDL-C, remnant-like particle cholesterol (RLP-C), and TG levels for each 10-year group in general Japanese population\(^{13}\). Framingham risk score (FRS) was established to estimate the 10-year individual risk of developing CHD in the Framingham Heart Study\(^{14, 15}\). However, the use of the FRS in some populations including the Japanese population resulted in an overestimation of the CHD risk because the Framingham cohort participants were mainly Caucasian\(^{16-18}\). In Japanese individuals, risk assessment chart was reported for estimating 10-year death probability from CHD-based NIPPON DATA 80 (NIPPON DATA risk), which was a 19-year follow-up study of a Japanese representative population since 1980\(^{19}\). The Hisayama risk prediction model (Hisayama risk) was established to estimate 10-year risk of cardiovascular disease (CVD) in a general Japanese population based on a cohort study of CVD in the Hisayama town\(^{20}\). Suita score was established to predict 10-year risk of CHD for Japanese population using the Suita study\(^{21}\). Suita score includes CKD as a coronary risk factor for Japanese population.

The aims of this study were to estimate the cholesterol levels of six lipoprotein classes in the serum of Japanese healthy men with age and to investigate the relationship between the lipoprotein profiles and three risk scores (NIPPON DATA risk, Hisayama risk, and Suita score) using the data obtained from healthy men.

### Materials and Methods

#### Subjects

The subjects of this study were the volunteers of the Tokyo Research Center of Tosoh Corporation, which obtained all volunteers’ assents with an informed consent form. The 161 healthy men (age, 25–64 years) without any medications were enrolled in this study. Hypertension was diagnosed based on systolic blood pressure (sBP) >140 mmHg and/or diastolic blood pressure (dBP) >90 mmHg (according to the Japanese Society of Hypertension Guidelines 2014). The concentration range in sera described the normal state of kidney as estimated glomerular filtration rate (eGFR) >60 mL/min/1.73 m\(^2\) (according to the Japan Society of Nephrology 2013). Dyslipidemia was diagnosed based on TG >150 mg/dL, HDL <40 mg/dL, and/or LDL >140 mg/dL (according to the Japan Atherosclerosis Society Guidelines 2012). A high fasting glucose level was defined as fasting plasma glucose (FPG) >110 mg/dL, and/or one of the diagnostic criteria for diabetes was hemoglobin A1c (HbA1c) >6.5% (according to the Japan Diabetes Society Guidelines 2013). The normal value ranges of hepatic enzymes (aspartate transaminase (AST) and alanine transaminase (ALT)) were <30 U/L according to the reference range determined by the Japan Society of Ningen Dock 2012.

#### Measurement

The serum AST, ALT, and creatinine (Cre) were measured according to an enzymatic method using Cica Liquid AST, Cica Liquid ALT, and Cica Liquid-S Cre (Kanto Chemical Co, Inc, Tokyo, Japan). The eGFR values were calculated using the new equation proposed by Japanese Society of Nephrology: eGFR for males (mL/min/1.73 m\(^2\)) = 194 × Cr\(^{-1.094}\) × Age\(^{-0.287}\) × 1.21, where Cr is the creatinine concentration in serum. TG, TC, HDL-C, and LDL-C levels were measured using Pureauto S TG-N, Choletest CHO, Choletest N HDL, and Choletest LDL (Sekisui Medical, Tokyo, Japan), respectively. FPG and HbA1c were measured using GA08 (A & T Corporation, Kanagawa, Japan) and HLC-723G8 (Tosoh Corporation, Tokyo, Japan), respectively.

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Fig. 1. Correlation between Lp (a) mass and Lp (a)-C

\[ y = 0.0563x + 2.001, \quad r = 0.6447, \quad n = 161, \quad P < 0.0001, \quad \text{respectively} \]
Non-HDL-C was calculated by subtracting HDL-C from TC. Lp (a)-mass was measured using Lp(a)-LATEX (Denka Seiken, Tokyo, Japan) based on latex-enhanced turbidimetric immunoassay.

Cholesterol levels of HDL, LDL, IDL, and VLDL were measured using HLC-7290P (Tosoh Corporation) based on AEX-HPLC method. Briefly, serum lipoproteins were separated into five lipoprotein classes (HDL, LDL, IDL, VLDL, and other) using a column by elution with step gradient of sodium perchlorate concentration. A column, which contained 2.5 µm of nonporous polymer-based gel with diethylaminoethyl ligands, and 2.5 mm ID × 10 mm in size, and a post-column reactor, which contained an enzymatic cholesterol reagent were used. It took 5.2 min to complete the assay of one sample. Cholesterol levels of two lipoprotein classes [CM and its remnant and Lp (a)] were measured using AEX-HPLC method described previously. Lp (a)-C measured by AEX-HPLC was correlated with Lp (a) mass measured by a latex-enhanced turbidimetric immunoassay (Fig. 1). The correlation coefficient was 0.6447 (*P < 0.0001, n = 161). 10-year CHD death probability (NIPPON DATA risk) was read from NIPPON DATA risk assessment.
Fig. 2. Comparison of the cholesterol concentration of six lipoproteins as per age using AEX-HPLC

The cholesterol levels of HDL, LDL, IDL, VLDL, CM, and Lp (a) were compared by age groups. The values indicate the mean ± standard deviation and they were shown in Table 1. All between-groups were compared using two-sample t-test or Mann-Whitney test according to F-test.

*: p < 0.05, **: p < 0.01, ***: p < 0.001

chart using gender, age, sBP, TC, FPG, and smoking tendency. 10-year risk score for CVD (Hisayama risk) was calculated by data of gender, age, LDL-C, HDL-C. 10-year risk score for CHD (Suita score) was calculated using data of gender, age, LDL-C, HDL-C, blood pressure, diabetes, smoker,
Table 2. Correlation coefficients between lipoprotein data by AEX-HPLC and clinical characteristics

| Lipoprotein data by AEX-HPLC | Number | Age | BMI | sBP | dBP | AST | ALT | FPG | HbA1c | Cr | eGFR | Suita score | NIPPON DATA risk | Hisayama risk |
|-----------------------------|--------|-----|-----|-----|-----|-----|-----|-----|-------|-----|------|-------------|------------------|---------------|
| Lp(a)-mass                  | 161    | -0.008 | -0.018 | 0.073 | 0.067 | 0.105 | 0.098 | 0.054 | -0.057 | 0.066 | -0.058 | 0.099 NS | -0.042 0.171 NS |
| non-HDL                     | 103    | 0.407 | 0.346 | 0.34 | 0.319 | 0.137 | 0.218 | 0.103 | 0.166 | -0.330 | 0.292 | 0.049 0.073 NS |

Not significant is indicated by NS.
Statistical significance was assessed using Spearman’s rank correlation coefficient (rS).
BMI, body mass index; sBP, systolic blood pressures; dBP, diastolic blood pressures; AST, Aspartate transaminase; ALT, Alanine transaminase; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; Cr, creatinine; eGFR, estimated glomerular filtration rate.

\*1: Suita score was calculated using excluded under 34 year age subjects.
\*2: NIPPON DATA risk and Hisayama risk were calculated using excluded under 39 year age subjects.

and the stage of CKD\(^20\). The stages of CKD were defined by eGFR levels\(^21\).

Statistical Analyses

Statistical analyses of the present data were performed by the Stat Flex Ver 6.0 software (Artech Co., Ltd., Osaka, Japan). The data were presented as mean ± standard deviation (SD). The data of multigroups by age were compared with one-way analysis of variance (one-way ANOVA) or Kruskal–Wallis test according to the Bartlett test. All between-groups were compared with two-sample t-test or Mann–Whitney test according to the F-test. The data of correlations were estimated by Spearman’s rank test. \(P\) values < 0.05 were considered significant. A stepwise multiple logistic regression analysis was performed to determine independent predictors of eGFR.

Results

Healthy study subjects (161 men) were classified into five groups by age (G1: 20–29 years, 28 men; G2: 30–39 years, 55 men; G3: 40–49 years, 39 men; G4: 50–59 years, 31 men; G5: 60–69 years, 8 men). The assay data of classified subjects are summarized in Table 1. In the data, other than lipid levels, sBP, dBP, ALT, eGFR, FPG, and HbA1c were significantly different among the age groups (one-way ANOVA). In the lipid data, TC, TG, non-HDL-C, and LDL-C measured by direct method were significantly different among the age groups. In lipoprotein data measured by AEX-HPLC, LDL-C, VLDL-C, and CM-C were significantly different among the age groups. In contrast, HDL-C, LDL-C, and Lp(a)-C were not significantly different among the age groups (Table 1). In the present study, the values of HDL-C, LDL-C, IDL-C, VLDL-C, CM-C, and Lp (a)-C in Japanese healthy men were 62.9 ± 12.7, 101.5 ± 18.1, 8.7 ± 2.5, 18.5 ± 7.5, 0.4 ± 0.9, and 2.6 ± 0.9 mg/dL, respectively. Fig. 2 showed the lipoprotein profiles between subgroup variations. HDL-C, Lp (a)-C, and Lp (a) mass had no significant difference among the age groups. Significant differences in LDL-C were observed between G1 and G2, G1 and G3, G1 and G4, G1 and G5, and G2 and G3.
G2 and G3. Significant differences in IDL-C were observed between G1 and G3 and G1 and G4. Significant differences in VLDL-C were observed between G1 and G2, G1 and G3, G1 and G4, and G2 and G4. Significant differences in CM-C were observed between G1 and G2, G1 and G3, G2 and G5, and G3 and G5. NIPPON DATA risk, Hisayama risk, and Suita score were also significantly different with age.

The correlations between the cholesterol levels of each lipoprotein measured by AEX-HPLC and the other clinical parameters are shown in Table 2. HDL-C was inversely related to body mass index (BMI), LDL-C was positively correlated with BMI, sBP, dBP, FPG, and Suita score and inversely correlated with eGFR. IDL-C was positively correlated with BMI and inversely correlated with eGFR. VLDL-C was positively correlated with BMI, sBP, and dBP and inversely correlated with eGFR. CM-C was positively correlated with BMI, sBP, NIPPON DATA risk, Hisayama risk, and Suita score. However, Lp (a)-C and Lp (a) mass did not correlate with any other clinical parameters. Non-HDL-C was positively correlated with BMI, sBP, dBP, FPG, and Suita score and inversely correlated with eGFR.

Next, we compared the cholesterol levels of each lipoprotein measured by AEX-HPLC and the other clinical parameters in subgroups classified based on Suita score (Table 3). Age, sBP, dBP, and HbA1c were significantly different by Suita score. LDL-C, TC, and non-HDL-C by usual method were significantly different by Suita score. In lipoprotein data measured by AEX-HPLC, the significant difference by Suita score was found only in CM-C.

Then, the study subjects (161 men) were classi-
fied into tertile groups based on eGFR levels [T1 (Low tertile; eGFR 60.2–76.3 mL/min/1.73 m³), T2 (Middle tertile; eGFR 76.6–88.4 mL/min/1.73 m³), and T3 (High tertile; eGFR 88.7–117.1 mL/min/1.73 m³)]. Although our present study used the healthy subjects without CKD, Suita score was calculated with eGFR, which was the biomarker of CKD. The data of classified subjects are shown in Table 4. In the data other than lipid levels, age, BMI, and sBP were significantly different among the eGFR groups. In lipid data, LDL-C, TC, TG, and non-HDL-C were significantly different among the eGFR groups. Lipoprotein data measured by AEX-HPLC, LDL-C, IDL-C, and VLDL-C were significantly different among the eGFR groups. Finally, the TG-rich lipoproteins and Lp (a)-C that contribute to eGFR were of interest. In a multiple regression analysis, we used BMI as a confounding factor correlated to the reduced eGFR (P = 0.0042). A multiple regression analysis indicated that VLDL-C value was significantly correlated with a reduced eGFR independent of BMI (Table 5).

**Discussion**

Previous studies have reported the relation of HDL-C, LDL-C, VLDL-C, or TC levels to age. A study reported by Arai et al. showed the age-specific means and SDs of serum TC, HDL-C, LDL-C, TG, and RLP-C levels by age group in the general Japanese

### Table 4. Basic data and lipid profiles of tertile groups by eGFR

| eGFR range (mL/min/1.73 m²) | T1 (Low tertile) | T2 (Middle tertile) | T3 (High tertile) | P |
|-----------------------------|------------------|---------------------|-------------------|---|
| Number (35 over *) | n = 53 (n = 42) | n = 54 (n = 44) | n = 54 (n = 17) | |
| Age | yrs | 45.3 ± 10.5 | 42.8 ± 10.2 | 34.3 ± 9.1 | < 0.0001 |
| BMI | kg/m² | 22.4 ± 1.8 | 22.0 ± 2.1 | 21.3 ± 2.3 | < 0.05 |
| sBP | mmHg | 115.6 ± 10.9 | 114.8 ± 10.4 | 110.8 ± 10.6 | < 0.05 |
| dbBP | mmHg | 70.3 ± 9.2 | 70.7 ± 8.6 | 67.9 ± 9.1 | NS |
| smoker | n, % | 12 (22.6%) | 10 (18.5%) | 9 (16.7%) | |
| AST | IU/L | 20.5 ± 3.79 | 19.0 ± 3.8 | 19.2 ± 3.5 | NS |
| ALT | IU/L | 18.55 ± 4.66 | 17.9 ± 4.5 | 17.1 ± 4.9 | NS |
| FPG | mg/dL | 88.0 ± 7.03 | 87.7 ± 6.4 | 85.2 ± 6.3 | NS |
| HbA1c | % | 5.3 ± 0.4 | 5.2 ± 0.3 | 5.2 ± 0.2 | NS |
| Cr | mg/dL | 0.935 ± 0.065 | 0.829 ± 0.060 | 0.745 ± 0.062 | < 0.0001 |
| eGFR | mL/min/1.73 m² | 70.7 ± 3.9 | 82.0 ± 3.2 | 98.5 ± 7.9 | < 0.0001 |
| Suita score* | % | 0.41 ± 0.87 | 0.59 ± 0.87 | 0.38 ± 0.73 | NS |

**Lipid Data by usual method**

| | mg/dL | |
|-----------------------------|------------------|---|
| HDL-C | 62.0 ± 12.8 | 66.1 ± 10.8 | 62.1 ± 12.0 | NS |
| LDL-C | 111.0 ± 18.3 | 110.2 ± 19.0 | 98.5 ± 21.1 | < 0.005 |
| TC | 188.2 ± 20.9 | 188.8 ± 23.5 | 172.7 ± 26.1 | < 0.0005 |
| TG | 81.1 ± 27.7 | 74.0 ± 24.7 | 60.7 ± 22.4 | < 0.0005 |
| Lp(a)-mass | 11.8 ± 11.0 | 10.1 ± 8.7 | 10.7 ± 11.6 | NS |
| non-HDL-C | 126.2 ± 20.1 | 122.7 ± 20.3 | 110.6 ± 22.7 | < 0.0005 |

**Lipoprotein Data by AEX-HPLC**

| | mg/dL | |
|-----------------------------|------------------|---|
| HDL-C | 60.9 ± 13.7 | 65.0 ± 10.8 | 62.7 ± 13.4 | NS |
| LDL-C | 105.0 ± 16.4 | 104.3 ± 18.4 | 95.4 ± 18.0 | < 0.01 |
| IDL-C | 9.4 ± 2.3 | 9.1 ± 2.3 | 7.8 ± 2.5 | < 0.005 |
| VLDL-C | 21.5 ± 8.0 | 19.0 ± 6.9 | 15.2 ± 6.3 | < 0.0001 |
| CM-C | 0.4 ± 1.1 | 0.3 ± 0.2 | 0.4 ± 1.0 | NS |
| Lp (a)-C | 2.7 ± 0.9 | 2.4 ± 0.7 | 2.7 ± 1.1 | NS |
| TC | 199.9 ± 22.2 | 200.0 ± 24.5 | 184.2 ± 27.9 | < 0.005 |

Values are presented as mean ± SD.

Stastical significance was assessed using Kruskal-Wallis test or one way ANOVA.

BMI, body mass index; sBP, systolic blood pressures; dbBP, diastolic blood pressures; AST, Aspartate transaminase; ALT, Alanine transaminase; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; Cr, creatinine; eGFR, estimated glomerular filtration rate.

*1 Suita score was calculated, excluded under 34 year age subjects.
were distributed within a relatively narrow and low range (2.6 ± 0.9 mg/dL). Lp (a)-C measured by AEX-HPLC was well correlated with Lp (a) mass (Fig. 1). Several methods to measure Lp (a)-C were previously reported. Nauck *et al.* reported direct determination of Lp (a)-C by ultracentrifugation and agarose gel electrophoresis. Sheman *et al.* reported a measurement method with lectin affinity to isolate Lp (a) from other lipoproteins. The comparison between these methods and our AEX-HPLC should be performed in the future. Some studies have suggested that high Lp (a) levels and the presence of smaller-molecular size of apo (a) are risk factors for atherosclerotic disease. The values of Lp (a)-C in healthy subjects showed little difference by age and no significant correlations with the clinical parameters associated with diabetes, CKD, or CHD (*Table 2*). These Lp (a)-C results of subjects with dyslipidemia remain to be defined.

It has been reported that individuals with CKD have increased levels of TG-rich lipoproteins. The CKD stage is defined using eGFR levels by the K/DOQI clinical practice guidelines. In this study, LDL-C, IDL-C, VLDL-C, and CM-C measured by AEX-HPLC were inversely correlated with eGFR (*Table 2*). LDL-C, IDL-C, and VLDL-C measured by AEX-HPLC were significantly different among eGFR tertile groups (*Table 4*). The multiple regression analysis showed that VLDL-C level was a significant explanatory factor for eGFR (*Table 5*). Shoji *et al.* showed that IDL-C and VLDL-C in hemodialysis patients were higher in patients with CKD than in healthy control. The VLDL receptor binds VLDL and participates in the clearance of VLDL from the circulation.

Previous study reported that VLDL receptor mRNA and protein are reduced in chronic renal failure rat models subjected to surgical partial nephrec-
were associated with atherosclerosis progression \cite{35, 36}. Thus, VLDL-C might be a good marker to predict renal dysfunction in healthy subjects. We need some case studies of patients with hypertension, diabetes mellitus, and dyslipidemia in addition to the present study to clarify that VLDL-C was also associated with eGFR in each diseased group.

Moreover, hepatic lipase gene expression is depressed in rats with experimental chronic renal failure (CRF), and lipoprotein lipase expression is down-regulated by CRF rats \cite{31, 32}. The downregulation of VLDL receptor pathway leads to an increase in plasma concentration and depressing clearance of VLDL in the early stage of CKD \cite{33}. Moreover, earlier atherosclerosis in kidneys is considered to induce intrarenal microvascular disease and renal injury in patients with CKD \cite{30}. Previous reports showed that IDL and VLDL were associated with atherosclerosis progression \cite{35, 36}. Thus, VLDL-C might be a good marker to predict renal dysfunction in healthy subjects. We need some case studies of patients with hypertension, diabetes mellitus, and dyslipidemia in addition to the present study to clarify that VLDL-C was also associated with eGFR in each diseased group.

Suita score was established to estimate the 10-year risk of CHD for Japanese population, and CKD is used for Suita score as a coronary risk factor \cite{21}. Suita score is much lower than FRS for the
10-year prediction of CHD events, which suggests that Suita score may be better than FRS to evaluate CHD risk for healthy Japanese population\(^2\). In Spearman’s rank test data, Suita core score was significantly correlated with age \((P<0.0001)\), sBP \((P<0.0001)\), dBP \((P<0.0001)\), FPG \((P<0.01)\), HbA1c \((<0.05)\), eGFR \((<0.05)\), and LDL-C \((<0.05)\), but not with HDL-C. CM-C was observed significant difference with Suita score \((P=0.003)\) as well as NIPPON DATA risk and Hisayama risk (Table 2). Furthermore, CM-C was significantly different in subgroups classified by Suita score (Table 3). Because LDL-C measured by homogeneous assay included IDL-C, LDL-C by AEX-HPLC might be not be significantly different unlike LDL-C by homogeneous assay. It was known that CM remnant was a predictor of atherosclerotic risk\(^{37-39}\) and CM remnant is relatively enriched in cholesteryl ester\(^{40}\). In this study, the cholesterol level in CM measured by AEX-HPLC mainly showed CM remnant cholesterol. It was considered that the remnant particles including CM remnant can enter the arterial wall and might increase the risk of atherogenesis\(^{41,42}\). LDL receptor-related proteins (LRPs) are a member of LDL receptor gene family and recognized some ligands including IDL and CM remnant\(^{31,43}\). Fujioka et al. suggested that CM remnants are taken up by via LDL receptor and LRP\(^{44}\). Elevated CM remnant cholesterol might represent the functional depression of LRP. Our data indicated that the CM-C by AEX-HPLC may be an independent marker for estimating CHD risk in healthy men. Nishimura et al. reported that the FRS overestimated the risk of CHD in Japanese subjects, especially in the non-CHD group, and the risks of hypertension and low HDL-C for males in the Suita cohort were weighted higher than the risk in the Framingham cohort\(^2\). For these reasons, IDL-C or VLDL-C might not be significantly correlated with Suita score in the present healthy subjects. Non-HDL-C is composed of LDL-C, IDL-C, VLDL-C, and CM-C\(^{45}\). We indicated the good correlation between Suita score and non-HDL-C in Table 2 and thought that the correlation was attributed to the associations between Suita score and LDL-C and CM-C. The present study has two limitations. One is that in the CM-C levels in Suita score, 1% subgroup was widely present \((0.7 \pm 1.7 \text{ mg/dL})\). Another was that in the present study, there was lack of subjects with CKD, and Suita score was characterized by CKD\(^2\). Then, we also analyzed using other risk scores in the Japanese population in addition to Suita score. NIPPON DATA risk was the risk assessment chart for estimating score of 10-year death probability from CHD-based NIPPON DATA 80\(^{19}\). The Hisayama risk was established to estimate 10-year risk of CVD-based Hisayama study\(^2\). NIPPON DATA risk and Hisayama risk were similar to Suita score and significantly different among the age groups. Moreover, significant difference was also observed with CM-C. In contrast, significant difference was not observed with non-HDL-C. To reconfirm the relationship between CM-C and Suita score or non-HDL-C and risk factors more clearly, we need a further large-scale study including some diseased subjects.

In conclusion, LDL-C, VLDL-C, and CM-C were significantly different as per age, and HDL-C, IDL-C, and Lp(a)-C were not significantly different in Japanese healthy men. VLDL-C value significantly correlated with eGFR. CM-C significantly correlated with Suita score. These results suggests that the increased serum VLDL-C concentration may contribute to an increased risk at early stages of renal dysfunction and that CM-C may serve as a useful marker for estimating CHD risk in Japanese healthy men although further studies are still needed.

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**Author Contribution**

D. Manita corrected data, contributed to the discussion, and wrote the manuscript. Y. Hirowatari and H. Yoshida designed the study protocol, contributed to the discussion and reviewed, and edited the manuscript.

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**Conflicts of Interest**

D. Manita is employee of TOSOH Corp (Tokyo, Japan).

Y. Hirowatari was employed with TOSOH Corp (Tokyo, Japan) until March 2015.

H. Yoshida declares no conflicts of interest in this study.

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