J Atheroscler Thromb, 2017; 24: 928-939.
http://doi.org/10.5551/jat.34728

Original Article

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

Daisuke Manita[1], Hiroshi Yoshida[2] and Yuji Hirowatari[3]

[1]Bioscience Division, TOSOH Corporation, Kanagawa, Japan
[2]Department of Laboratory Medicine, Jikei University Kashiwa Hospital, Chiba, Japan
[3]Laboratory Science, Department of Health Science, Saitama Prefectural University, Saitama, Japan

***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)[1, 2]. LDL-C is regarded as a primary target for the treatment of dyslipidemia because LDL-C is strongly associated with CHD risk[3, 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 Study[5]. Intermediate-density lipoprotein-cholesterol (IDL-C) was reported to have an association with the severity of CHD[6]. Furthermore, IDL-C is significantly increased in type Ⅲ hyperlipidemia[7]. Individuals with chronic kidney disease (CKD) are known to have high levels of triglyceride (TG)-rich lipoproteins including IDL and VLDL[8]. Raised IDL-C was associated with aortic sclerosis in hemodialysis patients[9].

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) method[10, 11]. Cholesterol levels of HDL, LDL, IDL, VLDL, and CM measured by

Address for correspondence: Yuji Hirowatari, Laboratory Science, Department of Health Science, Saitama Prefectural University, 820 San-nomiya, Kosigaya-shi, Saitama, Japan
E-mail: hirowatari-yuji@spu.ac.jp
Received: January 14, 2016
Accepted for publication: November 13, 2016

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.

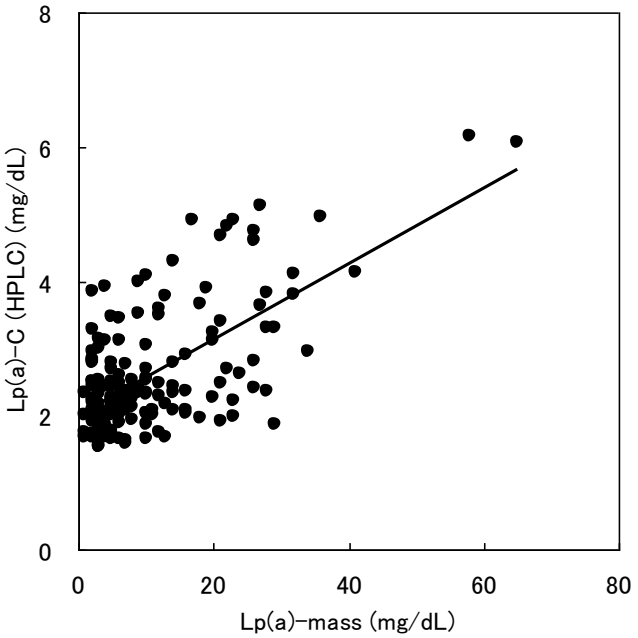

Fig. 1. Correlation between Lp (a) mass and Lp (a)-C
$y = 0.0563x + 2.001$, $r = 0.6447$, $n = 161$, and $P < 0.0001$, respectively

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) = $194 \times Cr^{-1.094} \times Age^{-0.287}$. 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),

Table 1. Basic data and lipid profiles of six groups by age

		Total $n=161$	20's $n=28$	30's $n=55$	40's $n=39$	50's $n=31$	60's $n=8$	*P*
Age	yeas	40.8 ± 11.0	27.0 ± 1.4	34.1 ± 3.0	44.5 ± 2.8	54.7 ± 2.8	62.8 ± 1.4	< 0.0001
BMI	kg/cm^2	21.9 ± 2.1	21.4 ± 2.4	21.7 ± 2.1	22.4 ± 1.9	22.2 ± 2.1	21.4 ± 2.1	NS
sBP	mmHg	113.7 ± 10.8	110.4 ± 9.5	110.8 ± 9.7	115.9 ± 11.1	118.3 ± 9.8	115.8 ± 16.1	< 0.01
dBP	mmHg	69.6 ± 9.0	65.1 ± 7.7	67.6 ± 7.3	71.2 ± 9.3	75.0 ± 9.5	72.3 ± 9.9	< 0.0001
smoker	n, %	31 (19.2%)	4 (14.3%)	11 (20.0%)	6 (15.4%)	9 (29.0%)	1 (12.5%)	
AST	IU/L	19.6 ± 3.7	18.4 ± 3.8	19.3 ± 3.4	20.1 ± 3.8	20.6 ± 4.4	18.6 ± 2.5	NS
ALT	IU/L	17.9 ± 4.7	16.6 ± 5.4	18.6 ± 4.3	18.7 ± 4.7	17.6 ± 4.2	14.1 ± 3.7	< 0.05
FPG	mg/dL	87.0 ± 6.6	83.5 ± 5.5	85.6 ± 6.0	88.6 ± 5.9	89.2 ± 8.0	91.8 ± 4.6	< 0.0005
HbA1c	%	5.2 ± 0.3	5.1 ± 0.3	5.1 ± 0.3	5.3 ± 0.3	5.4 ± 0.2	5.5 ± 0.2	< 0.0001
Cr	mg/dL	0.836 ± 0.100	0.843 ± 0.094	0.849 ± 0.118	0.827 ± 0.082	0.827 ± 0.090	0.796 ± 0.109	NS
eGFR	mL/min/1.73 m^2	83.9 ± 12.7	92.0 ± 11.1	86.6 ± 14.2	81.3 ± 9.3	77.4 ± 10.0	77.2 ± 10.6	< 0.0001
Risk Score								
Suita score[*1]	%	1.17 ± 0.47		1.00 ± 0.00	1.00 ± 0.00	1.39 ± 0.67	1.63 ± 0.74	< 0.0001
Nippon Data risk[*2]	%	0.60 ± 0.32			0.50 ± 0.00	0.53 ± 0.13	1.43 ± 0.54	< 0.0001
Hisayama risk[*2]	%	5.13 ± 2.44			3.23 ± 0.64	6.61 ± 1.71	9.20 ± 1.99	< 0.0001
Lipid Data by usual method								
HDL-C	mg/dL	63.4 ± 12.0	61.4 ± 8.8	63.5 ± 11.7	63.8 ± 12.8	64.0 ± 14.2	63.9 ± 13.2	NS
LDL-C	mg/dL	106.6 ± 20.2	92.6 ± 15.4	104.4 ± 20.3	111.6 ± 20.1	114.8 ± 18.8	117.9 ± 15.4	< 0.0001
TC	mg/dL	183.2 ± 24.6	165.6 ± 19.8	180.5 ± 25.7	189.1 ± 24.3	194.0 ± 19.6	196.1 ± 12.3	< 0.0001
TG	mg/dL	71.9 ± 26.3	58.2 ± 18.8	68.9 ± 27.2	75.0 ± 21.7	83.4 ± 28.5	81.4 ± 31.9	< 0.005
Lp(a)-mass	mg/dL	10.9 ± 10.5	9.1 ± 7.7	11.6 ± 9.5	9.3 ± 9.2	12.4 ± 12.8	15.2 ± 22.1	NS
non-HDL	mg/dL	119.8 ± 22.0	104.2 ± 16.4	116.2 ± 21.9	125.4 ± 21.4	130.0 ± 19.6	132.3 ± 18.0	< 0.0001
Lipoprotein Data by AEX-HPLC								
HDL-C	mg/dL	62.9 ± 12.7	60.6 ± 8.6	63.0 ± 12.7	64.2 ± 14.3	63.0 ± 14.1	61.7 ± 13.4	NS
LDL-C	mg/dL	101.5 ± 18.1	88.8 ± 13.2	99.5 ± 18.6	107.5 ± 18.8	107.6 ± 14.9	111.2 ± 12.9	< 0.0001
IDL-C	mg/dL	8.7 ± 2.5	7.7 ± 2.6	8.8 ± 2.7	9.2 ± 2.2	9.2 ± 1.9	8.2 ± 2.3	NS
VLDL-C	mg/dL	18.5 ± 7.5	14.1 ± 4.7	18.1 ± 7.7	20.1 ± 7.4	21.6 ± 7.9	18.3 ± 7.3	< 0.005
CM-C	mg/dL	0.4 ± 0.9	0.3 ± 0.2	0.2 ± 0.2	0.4 ± 1.2	0.5 ± 1.3	0.4 ± 0.2	< 0.01
Lp (a)-C	mg/dL	2.6 ± 0.9	2.6 ± 0.9	2.7 ± 1.3	2.6 ± 0.9	2.6 ± 1.0	3.0 ± 1.4	NS
TC	mg/dL	194.7 ± 25.9	174.1 ± 18.3	192.3 ± 28.1	204.0 ± 24.9	204.5 ± 19.8	202.8 ± 12.9	< 0.0001

Values are presented as mean ± SD.
Statical significance was assessed using Kruskal-Wallis test or one way ANOVA.
BMI, body mass index; sBP, systolic blood pressures; dBP, diastolic blood pressures; AST, Aspartate transaminase; ALT, Alanine transaminase; FPG, fasting plasma glucose; HbA1c, hemogrobin A1c; Cr, creatinine; eGFR, estimated glomerularfiltration rate.
[*1] Suita score was caluculated, excluded under 34 year age subjects.
[*2] NIPPON DATA risk and Hisayama risk were calculated using excluded under 39 year age subjects.

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-729LPII (Tosoh Corporation) based on AEX-HPLC method[22]. 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[11]. 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

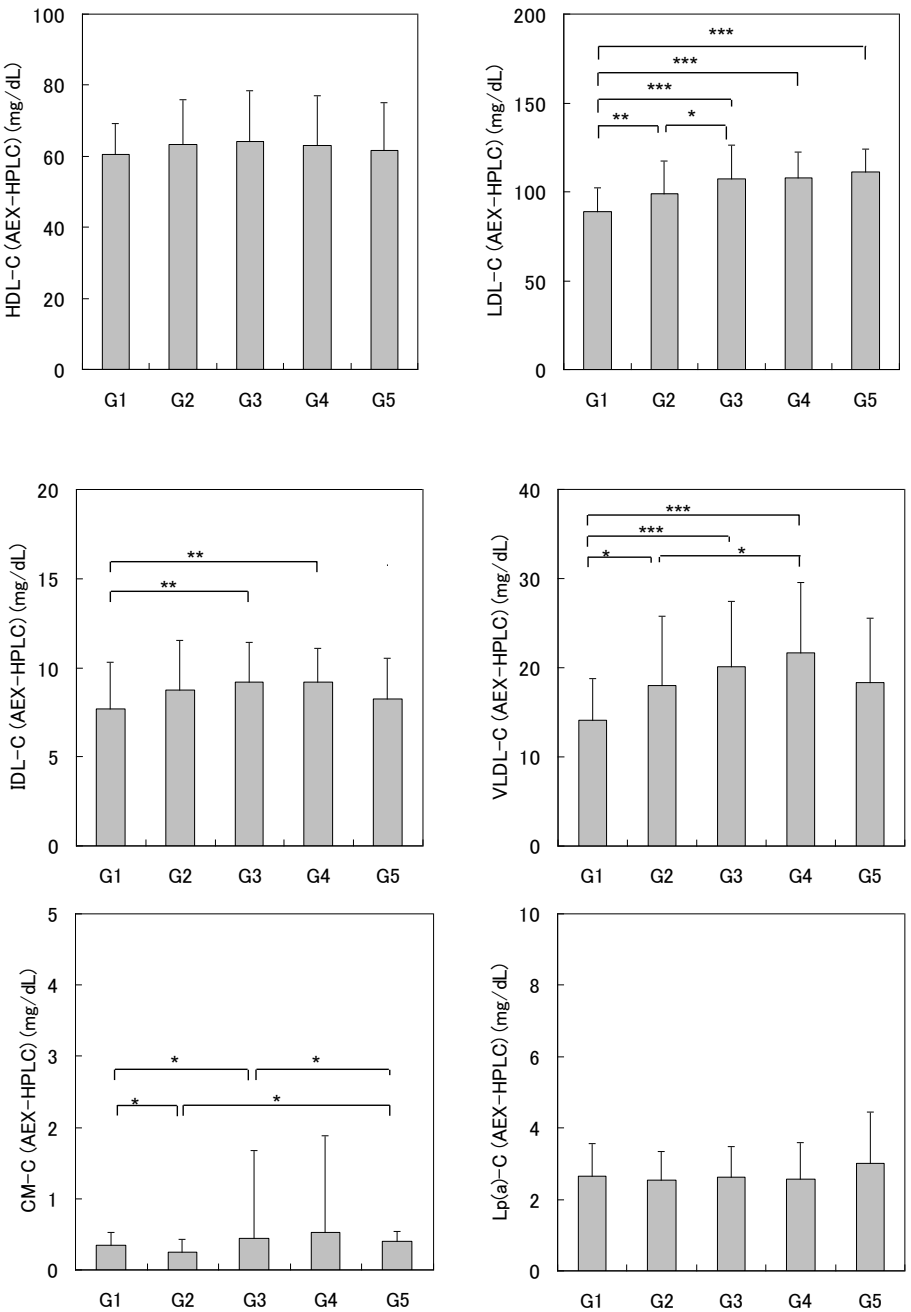

Fig. 2. Comparison of the cholesterol concentration of six lipoproteins as per age using AEX-HPLC

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[19]. 10-year risk score for CVD (Hisayama risk) was calculated by data of gender, age, sBP, smoker, LDL-C, and HDL-C[20]. 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

		Age	BMI	sBP	dBP	AST	ALT	FPG	HbA1c	Cr	eGFR	Suita score*1	NIPPON DATA risk*2	Hisayama risk*2
Number						161						103	77	77
Lp(a)-mass	rS	−0.008	−0.018	0.073	0.067	0.105	0.098	0.054	−0.057	0.066	−0.058	0.099	−0.042	0.171
	P values	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
non-HDL	rS	0.407	0.346	0.34	0.319	0.038	0.137	0.218	0.103	0.166	−0.330	0.292	0.049	0.073
	P values	<0.0001	<0.0001	<0.0001	<0.0001	NS	NS	<0.01	NS	<0.05	<0.0001	<0.005	NS	NS
Lipoprotein data by AEX-HPLC														
HDL-C	rS	0.056	−0.253	−0.083	−0.020	0.151	−0.013	0.110	0.043	−0.154	0.111	−0.043	−0.065	−0.057
	P values	NS	<0.005	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LDL-C	rS	0.364	0.302	0.308	0.286	0.044	0.087	0.187	0.108	0.088	−0.238	0.202	0.007	−0.044
	P values	<0.0001	<0.0001	<0.0001	<0.0005	NS	NS	<0.05	NS	NS	<0.005	<0.05	NS	NS
IDL-C	rS	0.213	0.193	0.102	0.064	−0.080	0.046	0.096	0.059	0.178	−0.258	−0.176	−0.123	−0.151
	P values	<0.01	<0.05	NS	NS	NS	NS	NS	NS	<0.05	<0.001	NS	NS	NS
VLDL-C	rS	0.294	0.239	0.203	0.170	−0.120	0.121	0.033	0.108	0.265	−0.349	0.013	−0.136	−0.078
	P values	<0.005	<0.005	<0.01	<0.05	NS	NS	NS	NS	<0.001	<0.0001	NS	NS	NS
CM-C	rS	0.083	0.186	0.180	0.119	0.017	0.081	0.093	0.030	0.134	−0.160	0.290	0.242	0.279
	P values	NS	<0.05	<0.05	NS	NS	NS	NS	NS	NS	<0.05	<0.005	<0.005	<0.005
Lp(a)-C	rS	−0.009	−0.001	0.121	0.080	0.220	0.154	0.095	−0.013	0.050	−0.055	0.016	0.041	0.036
	P values	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Not significant is indicaied by NS.
Statical significance was assessed using Speaman'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, hemogrobin A1c; Cr, creatinine; eGFR, estimated glomerularfiltration rate.
*1 Suita score was caluculated 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, IDL-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

Table 3. Basic data and lipid profiles of each Suita score groups

Suita score Number[*1]		0% *n* = 71	1% *n* = 19	2% *n* = 9	3% *n* = 4	*P*
Age	yeas	43.0 ± 5.7	54.8 ± 6.1	59.1 ± 3.7	58.0 ± 3.6	< 0.0001
BMI	kg/cm^2	22.1 ± 1.9	22.4 ± 2.7	21.9 ± 1.5	22.0 ± 2.7	NS
sBP	mmHg	113.2 ± 10.0	117.7 ± 12.6	124.0 ± 7.4	132.5 ± 2.5	< 0.0005
dBP	mmHg	69.0 ± 8.1	74.1 ± 10.5	81.6 ± 7.0	80.5 ± 7.7	< 0.0001
smoker	n, %	16 (23%)	4 (21%)	2 (22%)	3 (75%)	NS
AST	IU/L	19.5 ± 3.6	20.4 ± 4.4	21.2 ± 3.8	20.3 ± 1.0	NS
ALT	IU/L	18.9 ± 4.6	17.1 ± 5.2	17.7 ± 3.2	13.5 ± 2.4	NS
FPG	mg/dL	87.6 ± 6.3	91.8 ± 6.9	89.4 ± 6.2	91.8 ± 5.5	< 0.05
HbA1c	%	5.2 ± 0.3	5.4 ± 0.2	5.3 ± 0.1	5.3 ± 0.3	NS
Cr	mg/dL	0.840 ± 0.093	0.831 ± 0.101	0.827 ± 0.102	0.822 ± 0.100	NS
eGFR	mL/min/1.73 m^2	81.1 ± 10.3	76.7 ± 10.7	75.2 ± 10.1	75.8 ± 9.4	
Lipid Data by usual method						
HDL-C	mg/dL	63.2 ± 11.5	64.8 ± 14.0	66.9 ± 16.1	58.8 ± 10.3	NS
LDL-C	mg/dL	108.0 ± 19.3	119.7 ± 14.8	117.8 ± 13.7	127.5 ± 13.5	< 0.05
TC	mg/dL	184.9 ± 23.3	198.8 ± 12.6	199.0 ± 17.3	202.5 ± 6.8	< 0.05
TG	mg/dL	75.2 ± 25.3	80.2 ± 21.5	84.9 ± 35.1	93.3 ± 45.6	NS
Lp(a)-mass	mg/dL	9.9 ± 8.9	13.4 ± 15.2	15.9 ± 19.4	9.0 ± 5.6	NS
non-HDL-C	mg/dL	121.7 ± 20.9	134.0 ± 15.3	132.1 ± 16.2	143.8 ± 14.5	< 0.05
Lipoprotein Data by AEX-HPLC						
HDL-C	mg/dL	63.3 ± 12.8	63.3 ± 14.5	64.4 ± 14.4	55.9 ± 10.9	NS
LDL-C	mg/dL	103.7 ± 18.1	111.1 ± 13.4	110.6 ± 12.4	118.6 ± 9.2	NS
IDL-C	mg/dL	9.4 ± 2.1	8.6 ± 2.1	8.5 ± 2.2	8.8 ± 0.8	NS
VLDL-C	mg/dL	20.1 ± 7.4	20.4 ± 8.1	20.5 ± 9.4	21.2 ± 9.3	NS
CM-C	mg/dL	0.3 ± 0.9	0.7 ± 1.7	0.3 ± 0.1	0.5 ± 0.3	< 0.05
Lp (a)-C	mg/dL	2.6 ± 0.9	2.5 ± 1.0	2.9 ± 1.4	3.1 ± 0.8	NS
TC	mg/dL	199.5 ± 24.4	206.6 ± 15.6	207.1 ± 18.9	208.0 ± 9.3	< 0.05

Values are presented as mean ± SD.
Statical significance was assessed using Kruskal-Wallis test or one way ANOVA.
BMI, body mass index; sBP, systolic blood pressures; dBP, diastolic blood pressures; AST, Aspartate transaminase; ALT, Alanine transaminase; FPG, fasting plasma glucose; HbA1c, hemogrobin A1c; Cr, creatinine; eGFR, estimated glomerularfiltration rate.
[*1] Suita score was calculated, excluded under 34 year age subjects.

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-

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

eGFR range (mL/min/1.73 m^2)		T1 (Low tertile) 60.2-76.3	T2 (Middle tertile) 76.6-88.4	T3 (High tertile) 88.7-117.1	*P*
Number (35 over[*1])		*n* = 53 (*n* = 42)	*n* = 54 (*n* = 44)	*n* = 54 (*n* = 17)	
Age	yeas	45.3 ± 10.5	42.8 ± 10.2	34.3 ± 9.1	< 0.0001
BMI	kg/cm^2	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
dBP	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	20.51 ± 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 m^2	70.7 ± 3.9	82.0 ± 3.2	98.5 ± 7.9	< 0.0001
Suita score[*1]	%	0.41 ± 0.87	0.59 ± 0.87	0.38 ± 0.73	NS
Lipid Data by usual method					
HDL-C	mg/dL	62.0 ± 12.8	66.1 ± 10.8	62.1 ± 12.0	NS
LDL-C	mg/dL	111.0 ± 18.3	110.2 ± 19.0	98.5 ± 21.1	< 0.005
TC	mg/dL	188.2 ± 20.9	188.8 ± 23.5	172.7 ± 26.1	< 0.0005
TG	mg/dL	81.1 ± 27.7	74.0 ± 24.7	60.7 ± 22.4	< 0.0005
Lp(a)-mass	mg/dL	11.8 ± 11.0	10.1 ± 8.7	10.7 ± 11.6	NS
non-HDL-C	mg/dL	126.2 ± 20.1	122.7 ± 20.3	110.6 ± 22.7	< 0.0005
Lipoprotein Data by AEX-HPLC					
HDL-C	mg/dL	60.9 ± 13.7	65.0 ± 10.8	62.7 ± 13.4	NS
LDL-C	mg/dL	105.0 ± 16.4	104.3 ± 18.4	95.4 ± 18.0	< 0.01
IDL-C	mg/dL	9.4 ± 2.3	9.1 ± 2.3	7.8 ± 2.5	< 0.005
VLDL-C	mg/dL	21.5 ± 8.0	19.0 ± 6.9	15.2 ± 6.3	< 0.0001
CM-C	mg/dL	0.4 ± 1.1	0.3 ± 0.2	0.4 ± 1.0	NS
Lp (a)-C	mg/dL	2.7 ± 0.9	2.4 ± 0.7	2.7 ± 1.1	NS
TC	mg/dL	199.9 ± 22.2	200.0 ± 24.5	184.2 ± 27.9	< 0.005

Values are presented as mean ± SD.
Statical significance was assessed using Kruskal-Wallis test or one way ANOVA.
BMI, body mass index; sBP, systolic blood pressures; dBP, diastolic blood pressures; AST, Aspartate transaminase; ALT, Alanine transaminase; FPG, fasting plasma glucose; HbA1c, hemogrobin A1c; Cr, creatinine; eGFR, estimated glomerularfiltration rate.
[*1] Suita score was caluculated, excluded under 34 year age subjects.

fied into tertile groups based on eGFR levels [T1 (Low tertile; eGFR 60.2−76.3 mL/min/1.73 m^2), T2 (Middle tertile; eGFR 76.6−88.4 mL/min/1.73 m^2), and T3 (High tertile; eGFR 88.7−117.1 mL/min/1.73 m^2)]. Although our present study used the healthy subjects without CKD, Suita score was calculated with eGFR, which was the biomarker of CKD[21]. 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. In 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 5. A stepwise multiple regression analysis of the eGFR and various parameters ($n = 161$)

	Standard regression coefficients (β)	t value	P value
Basic data			
Body mass index (kg/m^2)	−0.7046	1.55254	0.1225
Lipoprotein data by anion-exchange HPLC			
HDL-C (mg/dL)	Not remain		
LDL-C (mg/dL)	−0.1106	1.96497	0.0512
IDL-C (mg/dL)	Not remain		
VLDL-C (mg/dL)	0.4738	3.56106	<0.0005
CM-C (mg/dL)	Not remain		
Lp(a)-C (mg/dL)	Not remain		

population, and HDL-C and LDL-C were measured by homogeneous assays[13]. Abbott *et al*. reported the cholesterol content of lipoprotein density classes measured by ultracentrifugation in the Framingham study during the period from 1971 to 1975 and the variations of HDL-C, LDL-C, and VLDL-C by age, gender, and use of hormone preparations[12]. **Fig. 3** shows the comparison levels of HDL-C, LDL-C, and VLDL-C by age group between the results of this study, the Japanese data reported by Arai *et al*.[13], and the Framingham study data[12]. Because LDL-C in the Japanese data by Arai *et al*. and the Framingham study data included IDL-C, we used the sum of LDL-C and IDL-C measured by AEX-HPLC as LDL-C for comparison[11, 22]. Compared with the Japanese data by Arai *et al*., given that the subjects in the survey data represented the civilian Japanese population having dyslipidemia, similar trends are observed in HDL-C and LDL-C. The concentration of HDL-C is lower in the Framingham study than in this study data measured by AEX-HPLC although similar trends are observed in HDL-C, LDL-C, and VLDL-C, while the concentration of LDL-C and VLDL-C was higher in the Framingham study than in this study data presumably because the subjects with hyperlipidemia were included in the Framingham study. The present study results need to be validated using large-scale study in the future.

In this study, Lp (a)-C levels did not show the significant variations by age group. Lp(a)-C levels seem to be little affected by age in healthy subjects because Lp (a) levels are profoundly affected by genetic factor[23]. We used the Lp(a)-LATEX (Denka Seiken, Tokyo, Japan) to measure Lp (a) mass, which was reported to be in harmony with the reference method and exhibited a positive bias for samples with apolipoprotein (a) {apo (a)} isoforms containing >25 kringle 4 domains[24]. In this study group, Lp (a)-C values 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[25]. Sheman *et al*. reported a measurement method with lectin affinity to isolate Lp (a) from other lipoproteins[26]. 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[26-28]. 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[8]. The CKD stage is defined using eGFR levels by the K/DOQI clinical practice guidelines[8]. 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[9]. The VLDL receptor binds VLDL and participates in the clearance of VLDL from the circulation[29].

Previous study reported that VLDL receptor mRNA and protein are reduced in chronic renal failure rat models subjected to surgical partial nephrec-

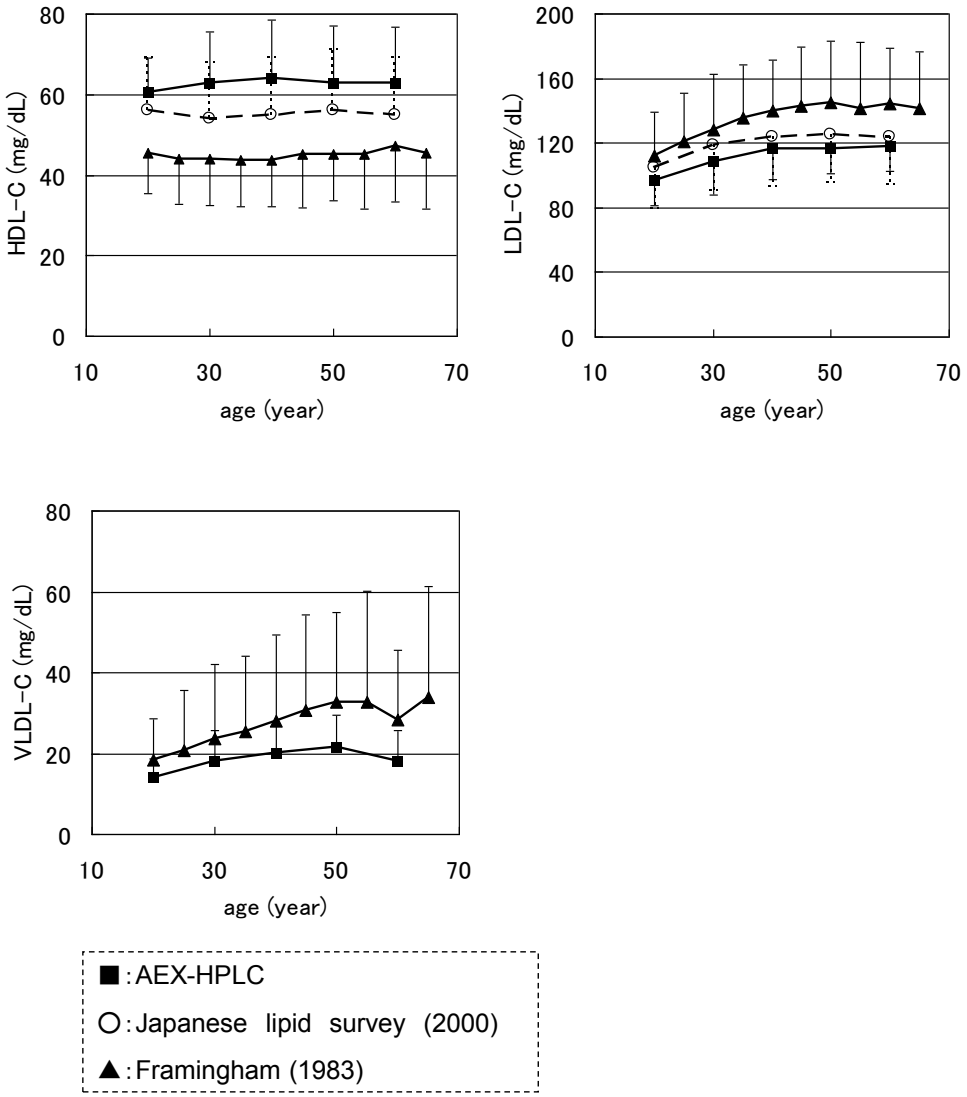

Fig. 3. Mean concentration of cholesterol in HDL, LDL (LDL + IDL), and VLDL in relation to age

Square indicates mean concentration of cholesterol measured by AEX-HPLC.
Circle indicates mean concentration of Japanese lipid survey reported by Arai *et al.* in 2005[13].
Triangle indicates mean concentration of the Framingham study reported by Abbott *et al.* in 1982[12].
Because LDL-C in these study data included IDL-C, we used the sum of LDL-C and IDL-C measured by AEX-HPLC as LDL-C for comparison.

tomy or parathyroidectomy[30].

Moreover, hepatic lipase gene expression is depressed in rats with experimental chronic renal failure (CRF), and lipoprotein lipase expression is downregulated by CRF rats[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[33]. Moreover, earlier atherosclerosis in kidneys is considered to induce intrarenal microvascular disease and renal injury in patients with CKD[34]. Previous reports showed that IDL and VLDL were associated with atherosclerosis progression[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[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[21]. 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 cholesterol 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[21]. 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 ± 1.7 mg/dL). Another was that in the present study, there was lack of subjects with CKD, and Suita score was characterized by CKD[21]. 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[20]. 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.

Acknowledgement

We would like to thank Ms Syuko Onodera for excellent technical assistance.

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.

Funding

This study was supported in part by grant-in-aid for Scientific Research (23591066 and 26461116) from Japan Ministry of Education, Culture, Sports, Science and Technology (H. Yoshida) and in part by the Tosoh Research Fund to D. Manita and Y. Hirowatari.

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.

References

1) Anderson KM, Castelli WP, Levy D: Cholesterol and mor-

tality. 30 years of follow-up from the Framingham study. JAMA, 1987; 257: 2176-2180
2) Barter P, Gotto AM, LaRosa JC, Maroni J, Szarek M, Grundy SM, Kastelein JJ, Bittner V, Fruchart JC: HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. N Engl J Med, 2007; 357: 1301-1310
3) Teramoto T, Sasaki J, Ishibashi S, Birou S, Daida H, Dohi S, Egusa G, Hiro T, Hirobe K, Iida M, Kihara S, Kinoshita M, Maruyama C, Ohta T, Okamura T, Yamashita S, Yokode M, Yokote K: Executive Summary of the Japan Atherosclerosis Society (JAS) Guidelines for the Diagnosis and Prevention of Atherosclerotic Cardiovascular Diseases in Japan -2012 Version. J. Atheroscler. Thromb, 2013; 20: 517-523
4) Stone NJ, Robinson JG, Lichtenstein AH, Merz CNB, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC, Watson K, Wilson PWF: 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults. J Am Coll Cardiol, 2014; 63: 2889-2934
5) Liu J, Sempos CT, Donahue RP, Dorn J, Trevisan M, Grundy SM: Non-high-density lipoprotein and very-low-density lipoprotein cholesterol and their risk predictive values in coronary heart disease. Am J Cardiol, 2006; 98: 1363-1368
6) Tatami R, Mabuchi H, Ueda K, Ueda R, Haba T, Kametani T, Ito S, Koizumi J, Ohta M, Miyamoto S, Nakayama A, Kanaya H, Oiwake H, Genda A, Takeda R: Intermediate-density lipoprotein and cholesterol-rich very low density lipoprotein in angiographically determined coronary artery disease. Circulation, 1981; 64: 1174-1184
7) Todo Y, Kobayashi J, Higashikata T, Kawashiri M, Nohara A, Inazu A, Koizumi J, Mabuchi H: Detailed analysis of serum lipids and lipoproteins from Japanese type Ⅲ hyperlipoproteinemia with apolipoprotein E2/2 phenotype. Clin Chim Acta, 2004; 348: 35-40
8) Kidney Disease Outcomes Quality Initiative (K/DOQI) Group: K/DOQI clinical practice guidelines for management of dyslipidemias in patients with kidney disease. Am J Kidney Dis, 2003; 41: 1-4, S1-91
9) Shoji T, Nishizawa Y, Kawagishi T, Kawasaki K, Taniwaki H, Tabata T, Inoue T, Morii H: Intermediate-density lipoprotein as an independent risk factor for aortic atherosclerosis in hemodialysis patients. J Am Soc Nerhol, 1998; 9: 1277-1284
10) Hirowatari Y, Yoshida H, Kurosawa H, Doumitu K, Tada N: Measurement of cholesterol of major serum lipoprotein classes by anion-exchange HPLC with perchlorate ion-containing eluent. J Lipid Res, 2003; 44: 1404-1412
11) Hirowatari Y, Yoshida H, Kurosawa H, Shimura Y, Yanai H, Tada N: Analysis of cholesterol levels in lipoprotein(a) with anion-exchange chromatography. J Lipid Res, 2010; 51: 1237-1243
12) Abbott RD, Garrison RJ, Wilson PW, Epstein FH, Castelli WP, Feinleib M, LaRue C: Joint distribution of lipoprotein cholesterol classes. The Framingham study. Arteriosclerosis, 1983; 3: 260-272
13) Arai H, Yamamoto A, Matsuzawa Y, Saito Y, Yamada N, Oikawa S, Mabuchi H, Teramoto T, Sasaki J, Nakaya N, Itakura H, Ishikawa Y, Ouchi Y, Horibe H, Kita T: Serum lipid survey and its recent trend in the general Japanese population in 2000. J Atheroscler Thromb, 2005; 12: 98-106
14) Wilson PWF, Castelli WP, Kannel WB: Coronary risk prediction in adults (the Framingham Heart Study). Am J Cardiol, 1987; 59: 91G-94G
15) Wilson PWF, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB: Prediction of Coronary Heart Disease Using Risk Factor Categories. Circulation, 1998; 97: 1837-1847
16) D'Agostino, Sr RB, Grundy S, Sullivan LM, Wilson P: Validation of the Framingham Coronary Heart Disease Prediction Scores. JAMA, 2001; 286: 180-187
17) Thomsen TF, McGee D, Davidsen M, Jørgensen T: A cross-validation of risk-scores for coronary heart disease mortality based on data from the Glostrup Population Studies and Framingham Heart Study. Int J Epidemiol, 2002; 31: 817-822
18) Hense H-W, Schulte H, Löwel H, Assmann G, Keil U: Framingham risk function overestimates risk of coronary heart disease in men and women from Germany--results from the MONICA Augsburg and the PROCAM cohorts. Eur Heart J, 2003; 24: 937-945
19) NIPPON DATA 80 Research Group: Risk Assesment Chart for Death From Cardiovascular Disease Based on a 19-Year Foiiow-up Study of a Japanese Representative Population. Circ J, 2006; 70: 1249-1255
20) Arima H, Yonemoto K, Doi Y, Ninomiya T, Hata J, Tanizaki Y, Fukuhara M, Matsumura K, Iida M, Kiyohara Y: Development and validation of a cardiovascular risk prediction model for Japanese: the Hisayama study.Hypertens Res, 2009; 32: 1119-1122
21) Nishimura K, Okamura T, Watanabe M, Nakai M, Takegami M, Higashiyama A, Kokubo Y, Okayama A, Miyamoto Y: Predicting Coronary Heart Disease Using Risk Factor Categories for a Japanese Urban Population, and Comparison with the Framingham Risk Score: The Suita Study. J Atheroscler Thromb, 2014; 21: 784-798
22) Manita D, Hirowatari Y, and Yoshida H: A rapid anion exchange chromatography for measurement of cholesterol concentrations in five lipoprotein classes and estimation of lipoprotein profiles in male volunteers without overt disease. Ann Clin biochem, 2015; 52: 638-646
23) Berglund L, Ramakrishnan R: Lipoprotein(a): An elusive cardiovascular risk factor. Arterioscler Thromb Vasc Biol, 2004; 24: 2219-2226
24) Marcovina SM, Albers JJ, Scanu AM, Kennedy H, Giaculli F, Berg K, Couderc R, Dati F, Rifai N, Sakurabayashi I, Tate JR, Steinmetz A: Use of a reference material proposed by the International Federation of Clinical Chemistry and laboratory medicine to evaluate analytical methods for the determination of plasma lipoprotein(a). Clin Chem, 2000; 46: 1956-1967
25) Nauck M, Winkler K, Wittmann C, Mayer H, Luley C, März W, Wieland H: Direct determination of lipoprotein(a) cholesterol by ultracentrifugation and agarose gel electrophoresis with enzymatic staining for cholesterol. Clin Chem, 1995; 41: 731-738
26) Seman LJ, DeLuca C, Jenner JL, Cupples LA, McNamara JR, Wilson PWF, Castelli WP, Ordovas JM, Schaefer EJ: Lipoprotein(a)-Cholesterol and Coronary Heart Disease

in the Framingham Heart Study. Clin Chem, 1999; 47: 1039-1046
27) Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D, Patsch W: Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation, 2001; 104: 1108-1113
28) Emerging Risk Factors Collaboration: Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. JAMA, 2009; 302: 412-423
29) Takahashi S, Warabayashi Y, Nakai T, Sakai J, Yamamoto T: Rabbit very low density lipoprotein receptor: a low density lipoprotein receptor-like protein with distinct ligand specificity. Proc Natl Acad Sci, 1992; 89: 9252-9256
30) Liang K, Oveisi F, Vaziri ND: Role of secondary hyperparathyroidism in the genesis of hypertriglyceridemia and VLDL receptor deficiency in chronic renal failure. Kidey Int, 1998; 53: 626-630
31) Klin M, Smogorzewski M, Ni Z, Zhang G, Massry SG: Abnormalities in hepatic lipase in chronic renal failure: Role of excess parathyroid hormone. J Clin Invest, 1996; 97: 2167-2173
32) Vaziri ND, Liang K: Down-regulation of tissue lipoprotein lipase in experimental chronic renal failure. Kidney Int, 1996; 50: 1928-1935
33) Vaziri ND: Dyslipidemia of chronic renal failure: the nature, mechanisms, and potential consequences. Am J Physiol Renal Physiol, 2006; 290: F262-F272
34) Chade AR, Lerman A, Lerman LO: Kidney in Early Atherosclerosis. Hypertension, 2005; 45: 104-1049
35) Mack WJ, Krauss R, Hodis HN: Lipoprotein subclasses in the Monitored Atherosclerosis Regression Study (MARS): treatment effects and relation to coronary angiographic progression. Arterioscler Thromb Vasc Biol, 1996; 16: 697-704
36) Hodis HN, Mack WJ, Dunn M, Liu C, Liu C, Selzer RH, Krauss RM: Intermediate-Density Lipoproteins and Progression of Carotid Arterial Wall Intima-Media Thickness. Circulation, 1997; 95: 2022-2026
37) Yu KC, Mamo JC: Chylomicron-remnant-induced foam cell formation and cytotoxicity: a possible mechanism of cell death in atherosclerosis. Clin Sci (Lond), 2000; 98: 183-192
38) Havel RJ: Postprandial hyperlipidemia and remnant lipoproteins. Curr Opin Lipidol, 1994; 5: 102-109
39) Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A: Postprandial lipoproteins and progression of coronary atherosclerosis. Atherosclerosis, 1994; 106: 83-97
40) Watts GF, Ooi EMM, Chan DC: Demystifying the management of hypertriglyceridaemia. Nat Rev Cardiol, 2013; 10: 648-661
41) Dominiczak MH: Apolipoprotein and Lipoproteins in Human Plasma. HANDBOOK OF LIPOPROTEIN TESTING 2nd Ed, ed by Rifai N, Warnick GR, Dominiczak MH, pp1-29, AACC Press, Washington DC, USA, 2000
42) Byrne CD: Triglyceride-rich lipoproteins: are links with atherosclerosis mediated by a procoagulant and proinflammatory phenotype? Atherosclerosis, 1999; 145: 1-15
43) Mokuno H, Brady S, Kotite S, Herz J, Havel RJ: Effect of the 39-kDa receptor-associated protein on the hepatic uptake and endocytosis of chylomicron remnants and low density lipoproteins in the rat, J Biol Chem, 1994; 269: 13238-13243
44) Fujioka Y, Cooper AD, Fong LG: Multiple processes are involved in the uptake of chylomicron remnants by mouse peritoneal macrophages. J Lipid Res, 1998; 39: 2339-2349
45) Pischon T, Girman CJ, Sacks FM, Rifai N, Stampfer MJ, Rimm EB: Non−high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. Circulation, 2005; 112: 3375-3383