reason that significance was not observed in the association of LDL-C with carotid plaque. The present study indicated that apoB48 level was strongly associated with carotid plaque. Even when LDL-C was within the normal range, an elevated apoB48 level would be a risk factor for atherosclerosis in type 2 DM.

In general, age, hypertension, history of smoking, and low HDL-C predict carotid atherosclerosis. However, these factors were not associated with the presence of carotid plaque in this study. The lack of association may be due to the characteristics and small number of the subjects. In our study, the proportion of age ≤45 years was 20%. This small proportion of young subjects may not have a statistical power, because carotid plaque area was reported to be strongly related to age, increased between 45 and 70 years of age markedly, and measurable plaque were more detected in >45-year subjects [28]. With respect to blood pressure, most of our subjects had normal blood pressure (the proportions of systolic blood pressure <135 mmHg and diastolic blood pressure <85 mmHg were 85% and 88%, respectively). Low HDL-C (<40 mg/dl) subjects were 20% in our study. These biases of the subjects may be the reason why the conventional atherosclerotic risk factors did not associated with the presence of carotid plaque in the present study. The lack of association between smoking habit and carotid plaque might be because we defined smoking as both current and past smoking, and did not account daily cigarette consumption.

ApoB48-containg lipoproteins were reported to be increased in production and decreased in clearance in type 2 DM [19], but little is known about its diurnal profile. The present study showed that the diurnal level of apoB48 fluctuated more than that of TG in type 2 DM, and was higher in plaque (+) than in plaque (−) throughout the day. The diurnal profile of apoB48 level would reflect the metabolism of chylomicron and its remnants, and high level of fasting apoB48 would indicate the accumulation of chylomicron remnants. These results suggest that apoB48 is more useful as a maker for atherogenic lipoprotein abnormality than TG. Campos et al. [29] reported the diurnal change in apoB48 level in normolipidemic subjects, indicating that the apoB48 level in both light and dense VLDL fraction were increased sharply after breakfast, increased slightly after lunch, and not increased immediately after dinner. In contrast, in the present study, serum apoB48 level was sharply increased after each meal. One reason for the inconsistent results may be the difference in the assay method for apoB48. Campos et al. evaluated apoB48 level in each lipoprotein fraction by SDS-PAGE, whereas we evaluated that in serum by ELISA. In addition, the difference in the subjects (normolipidemia vs. type 2 DM) might affect the discordance in the diurnal apoB48 profiles.

In conclusion, serum apoB48 level was strongly associated with the presence of carotid plaque in type 2 DM and was higher in the subjects with carotid plaque than in the subjects without the plaque throughout the day. The measurement of serum apoB48 may be useful to evaluate the risk of atherosclerosis. The present results warrant further studies, in large scale and prospective design, to confirm the significance of the measurement of apoB48.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.diabres.2008.04.028.

5. Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

[1] J.E. Hokanson, M.A. Austin, Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies, J. Cardiovasc. Risk 3 (1996) 213–219.

[2] N. Sarwar, J. Danesh, G. Eiriksdottir, G. Sigurdsson, N. Wareham, S. Bingham, et al., Triglycerides and the risk of coronary heart disease: 10, 158 incident cases among 262, 525 participants in 29 Western prospective studies, Circulation 115 (2007) 450–458.

[3] H. Iso, Y. Naito, S. Sato, A. Kitamura, T. Okamura, T. Sankai, et al., Serum triglycerides and risk of coronary heart disease among Japanese men and women, Am. J. Epidemiol. 153 (2001) 490–499.

[4] M.H. Criqui, G. Heiss, R. Cohn, L.D. Cowan, C.M. Suchindran, S. Bangdiwala, et al., Plasma triglyceride level and mortality from coronary heart disease, N. Engl. J. Med. 328 (1993) 1220–1225.

[5] L. Wilhelmsen, H. Wedel, G. Tibblin, Multivariate analysis of risk factors for coronary heart disease, Circulation 48 (1973) 950–958.

[6] B.G. Nordestgaard, M. Benn, P. Schnohr, A. Tybjaerg-Hansen, Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women, JAMA 298 (2007) 299–308.

[7] N. Mero, R. Malmström, G. Steiner, M.R. Taskinen, M. Syvänne, Postprandial metabolism of apolipoprotein B-48- and B-100-containing particles in type 2 diabetes mellitus: relations to angiographically verified severity of coronary artery disease, Atherosclerosis 150 (2000) 167–177.

[8] E. Meyer, H.T. Westerveld, F.C. de Ruyter Meijstek, M.M. van Greevenbroek, R. Rienks, H.J. van Rijn, et al., Abnormal postprandial apolipoprotein B-48 and triglyceride responses in normolipidemic women with greater than 70% stenotic coronary artery disease: a case–control study, Atherosclerosis 124 (1996) 221–235.

[9] F. Karpe, U. de Faire, M. Mercuri, M.G. Bond, M.L. Hellénius, A. Hamsten, Magnitude of alimentary lipemia is related to intima-media thickness of the common carotid artery in middle-aged men, Atherosclerosis 141 (1998) 307–314.

[10] N. Sakai, T. Sugimoto, K. Tsujii, H. Hiraoka, T. Nakamura, S. Yamashita, et al., Fasting serum apolipoprotein (apo) B-48 levels measured by a novel ELISA are a simple and useful marker for postprandial hyperlipidemia and predict coronary heart disease (CHD), Circulation 110 (Suppl. III) (2004), III-183.

[11] S. Boquist, G. Ruotolo, R. Tang, J. Björkegren, M.G. Bond, U. de Faire, et al., Alimentary lipemia, postprandial triglyceride-rich lipoproteins, and common carotid intima-media thickness in healthy, middle-aged men, Circulation 100 (1999) 723–728.

[12] R. Valero, A.M. Lorec, F. Paganelli, S. Beliard, C. Atlan, D. Lairon, et al., Fasting apoprotein B-48 level and coronary artery disease in a population without frank fasting hypertriglyceridemia, Metabolism 54 (2005) 1442–1447.
[13] K.G. Jackson, C.M. Williams, Apolipoprotein B-48: comparison of fasting concentrations measured in normolipidaemic individuals using SDS-PAGE, immunoblotting and ELISA, Atherosclerosis 176 (2004) 207–217.
[14] N. Sakai, Y. Uchida, K. Ohashi, T. Hibuse, Y. Saika, Y. Tomari, et al., Measurement of fasting serum apoB-48 levels in normolipidemic and hyperlipidemic subjects by ELISA, J. Lipid Res. 44 (2003) 1256–1262.
[15] Y. Uchida, Y. Kurano, S. Ito, Establishment of monoclonal antibody against human Apo B-48 and measurement of ApoB-48 in serum by ELISA method, J. Clin. Lab. Anal. 12 (1998) 289–292.
[16] S.M. Haffner, S. Lehto, T. Rönnemaa, K. Pyörälä, M. Laakso, Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction, N. Engl. J. Med. 339 (1998) 229–234.
[17] M.R. Taskinen, Diabetic dyslipidaemia: from basic research to clinical practice, Diabetologia 46 (2003) 733–749.
[18] A. Curtin, P. Deegan, D. Owens, P. Collins, A. Johnson, G.H. Tomkin, Elevated triglyceride-rich lipoproteins in diabetes. A study of apolipoprotein B-48, Acta Diabetol. 33 (1996) 205–210.
[19] J.C. Hogue, B. Lamarche, A.J. Tremblay, J. Bergeron, C. Gagné, P. Couture, Evidence of increased secretion of apolipoprotein B-48-containig lipoproteins in subjects with type 2 diabetes, J. Lipid Res. 48 (2007) 1336–1342.
[20] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (1972) 499–502.
[21] T. Teramoto, J. Sasaki, H. Ueshima, G. Egusa, M. Kinoshita, K. Shimamoto, et al., 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 disease for Japanese, J. Atheroscler. Thromb. 14 (2007) 155–158.
[22] C. Flood, M. Gustafsson, P.E. Richardson, S.C. Harvey, J.P. Segrest, J. Borén, Identification of the proteoglycan binding site in apolipoprotein B48, J. Biol. Chem. 277 (2002) 32228–32233.
[23] Y. Fujioka, A.D. Cooper, L.G. Fong, Multiple processes are involved in the uptake of chylomicron remnants by mouse peritoneal macrophages, J. Lipid Res. 39 (1998) 2339–2349.
[24] M.L. Brown, M.P. Ramprasad, P.K. Umeda, A. Tanaka, Y. Kobayashi, T. Watanabe, et al., A macrophage receptor for apolipoprotein B48: cloning, expression, and atherosclerosis, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 7488–7493.
[25] A. Kawakami, M. Tani, T. Chiba, K. Yui, S. Shinozaki, K. Nakajima, et al., Pitavastatin inhibits remnant lipoprotein-induced macrophage foam cell formation through ApoB48 receptor-dependent mechanism, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 424–429.
[26] S.D. Proctor, J.C. Mamo, Intimal retention of cholesterol derived from apolipoprotein B100- and apolipoprotein B48-containing lipoproteins in carotid arteries of Watanabe heritable hyperlipidemic rabbits, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 1595–1600.
[27] S. Pal, K. Semorine, G.F. Watts, J. Mamo, Identification of lipoproteins of intestinal origin in human atherosclerotic plaque, Clin. Chem. Lab. Med. 41 (2003) 792–795.
[28] J.D. Spence, M. Eliasziw, M. DiCicco, D.G. Hackam, R. Galil, T. Lohmann, Carotid plaque area. A tool for targeting and evaluating vascular preventive therapy, Stroke 33 (2002) 2916–2922.
[29] H. Campos, C. Khoo, F.M. Sacks, Diurnal and acute patterns of postprandial apolipoprotein B-48 in VLDL, IDL, and LDL from normolipidemic humans, Atherosclerosis 181 (2005) 345–351.

Original Article

Significance of Measuring Serum Concentrations of Remnant Lipoproteins and Apolipoprotein B-48 in Fasting Period

Itsuko Sato[1], Yuichi Ishikawa[2], Ai Ishimoto[2], Shiho Katsura[2], Atsushi Toyokawa[2], Fujio Hayashi[1], Seiji Kawano[1], Yoshio Fujioka[3], Shizuya Yamashita[4], and Shunichi Kumagai[1,5]

[1]Department of Clinical Laboratory, Kobe University Hospital, Kobe, Japan
[2]Faculty of Health Sciences, Kobe University Graduate School of Health Sciences, Kobe, Japan
[3]Faculty of Nutrition, Kobegakuin University, Kobe, Japan
[4]Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Osaka, Japan
[5]Department of Clinical Pathology and Immunology, Kobe University Graduate School of Medicine, Kobe, Japan

Aim: To characterize lipid profiles conveniently in the fasting period to detect postprandial hyperlipidemic subjects, we measured the concentrations of lipids, including remnant lipoproteins and apoB-48, before and after loading the test meal in 24 normolipidemic subjects.
Methods: We examined remnant-like particle-cholesterol and -triglyceride (RLP-C, RLP-TG) by the immune adsorption method, RemL-C by the newly developed homogeneous method, and apoB-48 by chemiluminescence enzyme immunoassay.
Results: After loading, TG, RemL-C, RLP-C, RLP-TG, and apoB-48 concentrations were elevated. Twenty subjects had only a slight elevation of TG (low TG group) after loading, while 4 subjects showed apparent increase of TG (more than 150 mg/dL, high TG group). In the fasting period, the high TG group had significantly higher serum concentrations of TG and RemL-C than the low TG group. Although not significant, RLP-C, RLP-TG and apoB-48 concentrations in the high TG group were also higher than in the low TG group. After loading, serum concentrations of TG, RemL-C, RLP-C, RLP-TG, and apoB-48 increased significantly more in the high TG group than in the low TG group.
Conclusion: In conclusion, TG, RemL-C, RLP-C, RLP-TG, and apoB-48 concentrations in the fasting period may be suitable for detecting postprandial hyperlipidemic subjects.

J Atheroscler Thromb, 2009; 16:12-20.

Key words; Postprandial hyperlipidemia, RLP-C, RemL-C, Small, dense LDL-C

Introduction

Since Zilversmit's proposal of the significance of postprandial hyperlipidemia, many studies have investigated the role of remnant lipoproteins in the pathogenesis of atherosclerosis, and have identified a delay in their removal from blood as an independent risk factor[1-6]. In fact, high concentrations of remnant-like particle (RLP)-cholesterol (RLP-C) predict coronary events in patients with CAD, independent of traditional coronary risk factors[7-9].

In normal humans, the postprandial hyperlipidemic period is about 4-6 hours, but in individuals with certain dyslipidemia, this period may be increased beyond 6 hours[3,4]. Of note, impaired removal of chylomicron remnants in the liver potentially induces longer retention times for these lipoproteins in blood circulation. Moreover, it is important to recognize that dietary lipid is transferred to various parts of the body via plasma lipoproteins after food ingestion.

In the postprandial period, we can observe the elevation of triglyceride (TG)-rich lipoproteins, which include chylomicrons, very-low-density lipoproteins

Address for correspondence: Yoshio Fujioka, Laboratory of Nutritional Physiology, Faculty of Nutrition, Kobegakuin University 518 Arise, Ikawadani-cho, Nishi-ku, Kobe, 651-2180, Japan
E-mail: fujioka@nutr.kobegakuin.ac.jp
Received: May 30, 2008
Accepted for publication: September 22, 2008

(VLDL), and their remnants, in the blood circulation. Nascent chylomicrons, synthesized by enterocytes, have a high TG-to-cholesterol mass ratio, and consist primarily of apolipoprotein (apo) B-48 and apoA-I [10, 11]. After acquiring apoC-II and apoE, chylomicrons bind to lipoprotein lipase (LPL), which induces lipolysis of TG in chylomicrons. TG depletion results in a size reduction, and is referred to as chylomicron remnants, or "remnants". Finally, chylomicron remnants are cholesteryl ester-rich and retain apoB-48 and apoE [12, 13]. VLDL produced by hepatocytes are also hydrolyzed by LPL like chylomicrons, and become VLDL remnants containing apoB-100 and apoE.

Although it has been difficult clinically to distinguish exogenous lipids (chylomicrons and their remnants) from endogenous lipids (VLDL and their remnants), it recently became possible to conveniently measure the serum B-48 concentration [14]. In the present study, to characterize the lipid profiles conveniently in the fasting period to detect postprandial hyperlipidemic subjects, we measured the concentrations of lipids, including remnant lipoproteins and apoB-48, before and after loading the test meal in normolipidemic subjects.

Subjects and Methods

Subjects and Physical Examination

We recruited healthy subjects [n=24; male/female, 11/13; mean ± standard deviation (SD), 21.5 ± 1.2 years old] who had never been treated or taken any drugs at least 3 months before the study. All subjects gave their informed consent to participate in the study. The study protocol was carried out according to the Declaration of Helsinki.

Blood pressure was measured twice by the same observer using a standard mercury sphygmomanometer after the subject had rested in a supine position for 30 min. Waist circumference of subjects was measured [15]. Body mass index (BMI) was calculated by dividing body weight by the square of the height (kg/m^2).

Study Protocol

We used a test meal containing carbohydrate, fat, and protein, which was developed for the assessment of both postprandial hyperglycemia and hyperlipidemia by the Japanese Diabetes Society (Test meal A). This test meal consists of cream of chicken soup, biscuit, and custard pudding. The total calories is 450 kcal, including carbohydrate 57.6 g (51.4% in energy balance), protein 17.2 g (15.3%), fat 16.6 g (33.3%), which is a slightly higher percentage of fat than in the usual Japanese breakfast (20–25%). Blood samples were obtained at 9–10 AM after a 12-hour fast and 1, 2, 4, 6, and 8 hours after ingestion of the test meal.

Blood Sampling and Analysis

Serum concentrations of TG, total cholesterol, high-density lipoprotein (HDL)-cholesterol (HDL-C) and low-density lipoprotein (LDL)-cholesterol (LDL-C) were determined by enzymatic methods (Kyowa Medex, Tokyo, Japan): plasma oxidized LDL concentration by enzyme-linked immunosorbent assay (Kyowa Medex); serum concentrations of apoA-I, apoA-II, apoB, apoC-II, apoC-III, and apoE by turbidimetric immunoassay methods (Nittobo, Tokyo, Japan); serum apoB-48 concentration by chemiluminescence enzyme immunoassay (Fujirebio, Tokyo, Japan) [14]; serum small, dense-LDL-cholesterol (sd-LDL-C) concentration by the precipitation method (Denka Seiken, Tokyo, Japan) [16]; serum concentrations of RLP-C and RLP-TG by the immune adsorption method (JIMRO II, Otsuka Pharmaceutical, Tokyo, Japan) [17]; serum remnant lipoprotein cholesterol (RemL-C) concentration by homogenenous assay (MetaboLead RemL-C, Kyowa Medex) [18]; serum high sensitivity C-reactive protein (hs-CRP) concentration by nephelometry method (Dade Behring, Deerfield, IL); plasma glucose concentration and glycosylated hemoglobin A1c (HbA1c) by HPLC; and serum insulin concentrations by enzyme immunoassay, respectively.

Agarose Gel Electrophoresis Analysis

Samples were subjected to lipoprotein analysis using agarose gel electrophoresis (Rapid Electrophoresis; Helena Laboratories, Beaumont, Texas), with 15 min of electrophoresis at 400 volts and 20℃. After staining with cholesterol and TG reagent, elution profiles were analyzed by an automatic densitometer, Chol/Trig Combo™ (Helena Kenkyusho, Saitama, Japan) [19]. Contents of cholesterol and TG in each fraction were calculated with total lipids and the area under the curve according to the report by Kido *et al.* [20]. Moreover, the ratios of cholesterol to TG in HDL and LDL fractions were calculated and compared with those in healthy volunteers reported previously (mean ± SD; HDL, 5.8 ± 2.0; LDL, 4.9 ± 1.3) [21].

Statistical Analysis

Values are expressed as the mean ± SD. Statistical significance of data was evaluated using either the Mann-Whitney U-test or Welch's t-test. Correlations between apoB-48 and other parameters were calculated using the formula for Pearson's correlation coefficient. Responses to the test meal were compared by analysis of variance (ANOVA) for repeated measures.

Table 1. Glucose and lipid parameters before and after loading the test meal

	0	1h	2h	4h	6h	8h
Plasma glucose (mg/dL)	89.4±4.7	104.5±19.2**	89.2±11.5	85.3±4.8**	85.2±4.5**	85.1±5.0**
Insulin (μU/mL)	6.1±2.9	53.7±27.3**	24.6±16.4**	5.1±2.2	4.1±1.3*	3.7±1.4*
TG (mg/dL)	65.6±25.5	86.9±38.2*	95.7±47.4*	77.1±32.6	60.7±19.2	52.2±16.8*
TC (mg/dL)	182.6±32.2	181.5±33.8	179.8±32.4	181.4±30.8	184.2±31.7	189.5±33.3
LDL-C (mg/dL)	100.0±25.6	97.8±25.7	96.8±25.4	97.9±24.4	101.1±25.2	104.3±26.2
HDL-C (mg/dL)	70.0±14.3	67.8±14.2	67.2±13.1	68.0±14.1	70.1±14.9	72.5±15.2
RemL-C (mg/dL)	3.5±1.6	3.9±1.9	4.0±2.1	3.7±2.1	3.1±1.3	2.9±1.1
RLP-C (mg/dL)	3.1±1.2	4.4±2.0*	4.7±2.6*	3.8±1.7	3.0±1.3	2.8±0.9
RLP-TG (mg/dL)	15.8±2.6	24.8±14.6*	30.4±23.6*	20.1±8.7	15.2±0.7*	15.1±0.2*
Sd-LDL-C (mg/dL)	21.4±8.9	17.3±5.8	17.1±6.9	17.0±5.7	17.5±5.9	17.8±5.8
Oxidized LDL (U/mL)	6.7±4.9	6.1±4.4	6.6±5.5	6.5±4.8	6.8±4.7	7.1±4.9
ApoA-I (mg/dL)	164.0±27.0	161.8±28.0	161.3±25.0	162.5±26.7	165.5±26.7	168.2±28.1
ApoA-II (mg/dL)	38.9±7.1	38.5±7.4	38.0±6.9	38.5±7.0	38.7±7.1	39.3±7.1
ApoB (mg/dL)	67.5±14.2	66.3±14.8	66.2±14.0	66.8±13.6	68.5±13.9	70.5±14.2
ApoB-48 (μg/mL)	3.2±2.1	6.2±3.1**	6.1±3.4**	5.2±2.9**	3.6±2.1	3.0±1.7
ApoC-II (mg/dL)	3.1±1.1	3.2±1.2	3.2±1.2	3.2±1.1	3.2±1.1	3.2±1.1
ApoC-III (mg/dL)	9.4±2.5	9.9±2.6	9.5±2.6	9.3±2.4	9.1±2.3	9.3±2.4
ApoE (mg/dL)	4.6±1.0	4.5±1.0	4.4±1.0	4.4±1.0	4.3±0.9	4.4±0.9

Values are expressed as the mean±SD, *$p<0.05$, **$p<0.01$ (vs. 0 time) by Mann-Whitney *U*-test. TG: triglyceride, TC: total cholesterol, LDL-C: low-density lipoprotein-cholesterol, HDL-C: high density lipoprotein-cholesterol, RemL-C: remnant lipoprotein cholesterol measured with "Metabo-Lead RemL-C", RLP-C: remnant-like particle-cholesterol measured with "JIMRO II", RLP-TG: remnant-like particle-triglyceride, Sd-LDL-C: small, dense-LDL-cholesterol, Apo: apolipoprotein.

Data under the threshold of RLP-C (<2.0 mg/dL) or RLP-TG (<15 mg/dL) were treated as 2.0 mg/dL or 15 mg/dL, respectively. Statistical analysis was performed using Stat Flex ver.5.0 software (Artec, Osaka, Japan). Two-tailed values of $p<0.05$ were considered significant.

Results

Characteristics of Subjects

Subject characteristics are as follows (mean±SD): BMI, 20.7±1.7 kg/m^2; waist circumferences, 72.3±4.2 cm in men and 65.7±4.5 cm in women; HbA_{1c}, 4.9±0.2%; hsCRP, 0.04±0.02 mg/dL.

Fasting and Postprandial Concentrations of Lipids in Total Subjects

Table 1 shows the changes of lipid concentrations before and after loading the test meal in all subjects. In the fasting period (time 0), concentrations of all parameters were within normal limits or low ranges; however, there were significant correlations between apoB-48 concentration and TG, RemL-C, RLP-C, RLP-TG, apoC-II, or apoC-III concentration (**Table 2**), indicating that intestine-derived lipoproteins were present in the circulation and had characteristics of remnants even in the fasting period in normolipidemic subjects.

After loading the test meal, TG, RLP-C, RLP-TG, and apoB-48 concentrations elevated significantly compared with before loading (**Table 1**). TG, RLP-C, and RLP-TG concentrations peaked at 2 hours, and were restored to the baseline within 4 hours. ApoB-48 concentrations peaked at 1 hour, and returned to basal levels at 6 hours. RemL-C concentrations also peaked at 2 hours and were restored within 6 hours, but this elevation had no significance. On the other hand, the concentrations of TC, HDL-C, LDL-C, sd-LDL-C, oxidized LDL, apoA-I, apoA-II, apoB, apoC-II, apoC-III, and apoE were not elevated. Sd-LDL-C concentrations decreased below the basal levels during the study without statistical significance.

Comparison of Fasting Lipid Concentrations between High and Low TG Groups

In the results of the loading test, we noticed that some subjects showed apparent increases of TG at 2 hours as peak values, and others showed only a slight elevation. We therefore established two groups by TG values at 2 hours, and designated subjects with <150 mg/dL of TG ($n=20$) as the low TG group and subjects with >150 mg/dL of TG ($n=4$) as the high TG

Table 2. Correlation between apoB-48 concentration and other parameters in fasting period

	r	p
Plasma glucose (mg/dL)	0.236	0.2661
Insulin (μU/mL)	0.042	0.8448
TG (mg/dL)	0.791	<0.0001
TC (mg/dL)	0.287	0.1743
LDL-C (mg/dL)	0.301	0.1526
HDL-C (mg/dL)	−0.081	0.7065
RemL-C (mg/dL)	0.811	<0.0001
RLP-C (mg/dL)	0.768	<0.0001
RLP-TG (mg/dL)	0.745	<0.0001
Sd-LDL-C (mg/dL)	0.367	0.0776
Oxidized LDL (mg/dL)	−0.150	0.4832
ApoA-I (mg/dL)	0.016	0.9395
ApoA-II (mg/dL)	0.290	0.1693
ApoB (mg/dL)	0.282	0.1820
ApoC-II (mg/dL)	0.689	0.0002
ApoC-III (mg/dL)	0.534	0.0071
ApoE (mg/dL)	0.189	0.3764
hs-CRP (mg/L)	0.253	0.2321

Correlations between apoB-48 and other parameters were calculated using the formula for Pearson's correlation coefficient. TG: triglyceride, TC: total cholesterol, LDL-C: low-density lipoprotein-cholesterol, HDL-C: high density lipoprotein-cholesterol, RemL-C: remnant lipoprotein cholesterol measured with "MetaboLead RemL-C", RLP-C: remnant-like particle-cholesterol measured with "JIMRO II", RLP-TG: remnant-like particle-triglyceride, Sd-LDL-C: small, dense-LDL-cholesterol, Apo: apolipoprotein, hs-CRP: high sensitivity-C-reactive protein.

Table 3. Comparison of clinical characteristics and fasting concentrations of glucose and lipid parameters between two groups

	High TG group (n=4)	Low TG group (n=20)	p
Age (years old)	22.0±2.5	21.4±0.9	0.6695
BMI (kg/m²)	22.0±2.2	20.4±1.5	0.2975
Abdominal circumference (cm)	73.5±5.7	67.8±5.2	0.1334
Hs-CRP (mg/dL)	0.06±0.02	0.04±0.02	0.1655
HbA1c (%)	5.0±0.2	4.8±0.2	0.3018
Plasma glucose (mg/dL)	83.0±4.8	91.0±3.6	0.0719
Insulin (μU/mL)	6.3±2.6	6.1±3.0	0.9060
TG (mg/dL)	109.5±15.8	56.9±16.1	0.0044
TC (mg/dL)	196.5±14.6	179.9±34.3	0.1460
LDL-C (mg/dL)	110.3±16.6	98.0±26.8	0.2982
HDL-C (mg/dL)	67.5±26.7	70.5±11.5	0.8477
RemL-C (mg/dL)	5.9±1.3	3.0±1.2	0.0148
RLP-C (mg/dL)	4.9±1.4	2.8±0.8	0.0694
RLP-TG (mg/dL)	19.5±5.4	15.6±2.7	0.2744
Sd-LDL-C (mg/dL)	30.7±13.0	17.9±5.6	0.1602
Oxidized LDL (U/mL)	4.9±1.7	7.0±5.2	0.1630
ApoA-I (mg/dL)	163.8±49.1	164.0±22.5	0.9930
ApoA-II (mg/dL)	44.0±10.4	37.9±6.0	0.3609
ApoB (mg/dL)	75.8±10.2	65.8±14.5	0.1865
ApoB-48 (μg/mL)	6.1±2.6	2.6±1.5	0.0957
ApoC-II (mg/dL)	3.6±1.1	2.9±1.1	0.3734
ApoC-III (mg/dL)	11.3±3.7	9.1±2.1	0.3531
ApoE (mg/dL)	5.1±1.1	4.5±0.9	0.4477

Values are expressed as the mean±SD, Welch's *t* test. High group: the peak triglyceride (TG) concentration ≥150 mg/dL, low group: peak TG concentration <150 mg/dL, BMI: body mass index, HbA1c: glycosylated hemoglobin A1c, TC: total cholesterol, RemL-C: remnant lipoprotein cholesterol measured with "MetaboLead RemL-C", RLP-C: remnant-like particle-cholesterol measured with "JIMRO II", RLP-TG: remnant-like particle-triglyceride, Sd-LDL-C: small, dense-LDL-cholesterol, Apo: apolipoprotein.

group. The peak concentrations of TG in the low TG group were 78.4±27.6 mg/dL, whereas those in the high TG group were 182.5±33.7 mg/dL.

First, we compared the profiles and lipid parameters in the fasting period between the two groups (**Table 3**). The high TG group had significantly higher serum concentrations of TG and RemL-C than the low TG group. Although not significant, RLP-C, RLP-TG and apoB-48 concentrations in the high TG group were also higher than in the low TG group. There were no significant differences in the profiles, including age, BMI, and abdominal circumference between the two groups.

Comparison of Postprandial Concentrations between High and Low TG Groups

Fig. 1 demonstrates the sequential changes of parameters in lipids before and after loading the test meal in each group. Serum concentrations of TG, RemL-C, RLP-C, RLP-TG, and apoB-48 significantly increased more in the high TG group than in the low TG group, especially from 1 to 4 hours. In contrast, although sd-LDL-C concentrations were significantly higher in the high TG group, they gradually decreased after loading. There were no significant differences in serum concentrations of TC, LDL-C, HDL-C, and other apolipoproteins. Concentrations of glucose, insulin, and oxidized LDL also did not alter (data not shown).

Analysis of Lipid by Electrophoretogram

Fig. 2 demonstrates representative cases of densitometric scanning patterns of electrophoretogram and lipid data before and after loading the test meal in the high and low TG groups. Sample A belongs to the high TG group. Fractions of chylomicrons and VLDL

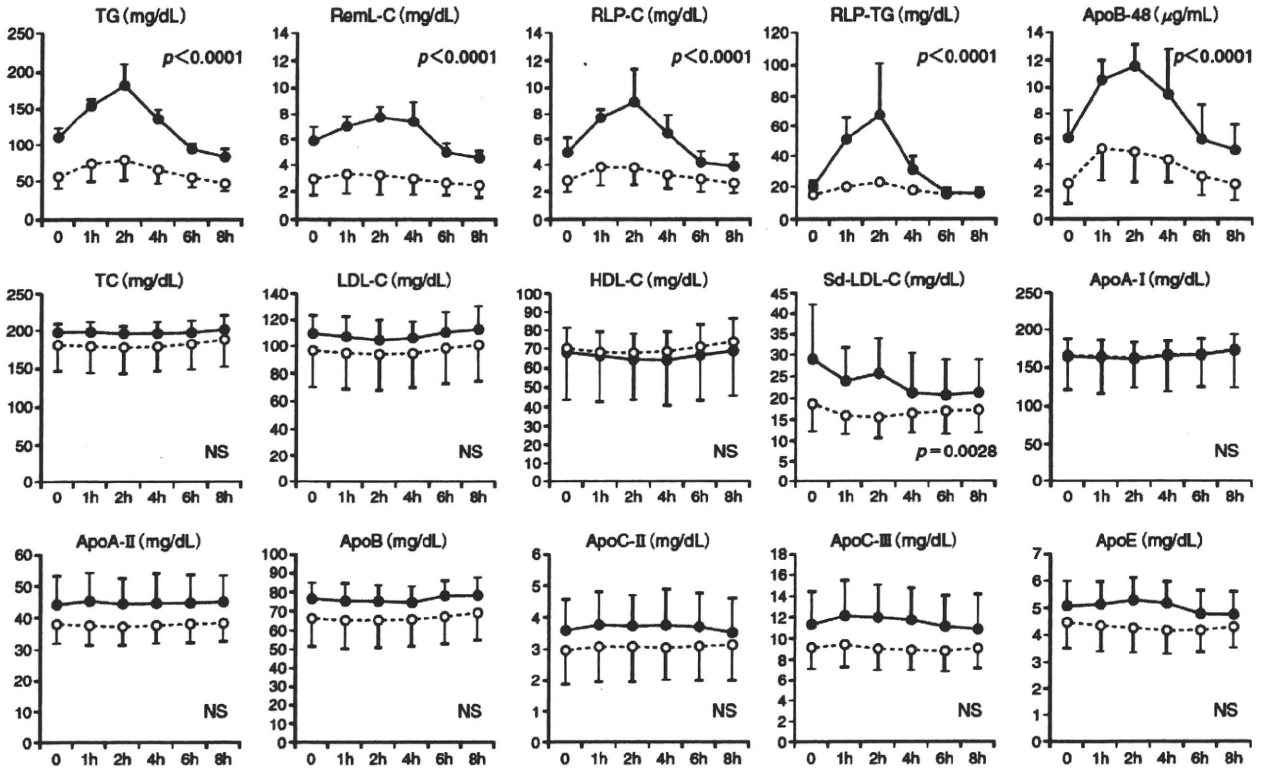


Fig. 1.

Changes in the levels of glucose and lipid parameters in subjects with <150 mg/dL (low TG group) and >150 mg/dL triglyceride concentration (high TG group) before and after loading the test meal. Responses to the test meal were compared by analysis of variance (ANOVA) for repeated measures. Open circle and broken line: low TG group, closed circle and black line: high TG group, TG: triglyceride, RemL-C: remnant lipoprotein cholesterol measured with "MetaboLead RemL-C", RLP-C: remnant-like particle-cholesterol measured with "JIMRO II", RLP-TG: remnant-like particle-triglyceride, TC: total cholesterol, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, sd-LDL-C: small, dense-LDL-cholesterol.

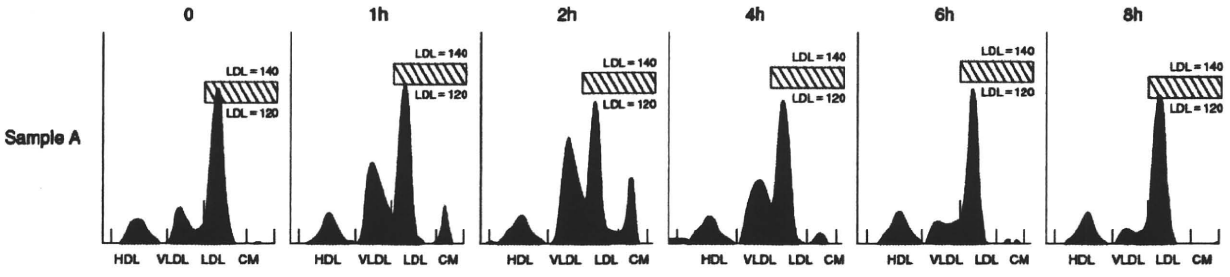
increased 1 hour after loading, peaked at 2 hours, and gradually decreased. Sample B belongs to the low TG group. We observed a slight increase of the chylomicron fraction 2 hours after loading. Thus, the peak values of TG and lipoprotein profiles in the postprandial period were different between the two cases, although fasting TG concentrations in both cases were around 80 mg/dL.

Fig. 3 shows the changes in TG concentrations of chylomicrons and VLDL, and the ratios of cholesterol to TG in HDL and LDL before and after loading the test meal. In the left panel of sample A, the higher the TG concentrations of chylomicrons and VLDL, the lower the ratios of cholesterol to TG in HDL and LDL decreased to levels around the mean-2SD of normal subjects (1.8, 2.3, respectively), as previously reported[21]. In the right panel of sample B, TG contents of chylomicrons and VLDL did not increase, and the ratios of cholesterol to TG in HDL and LDL decreased slightly.

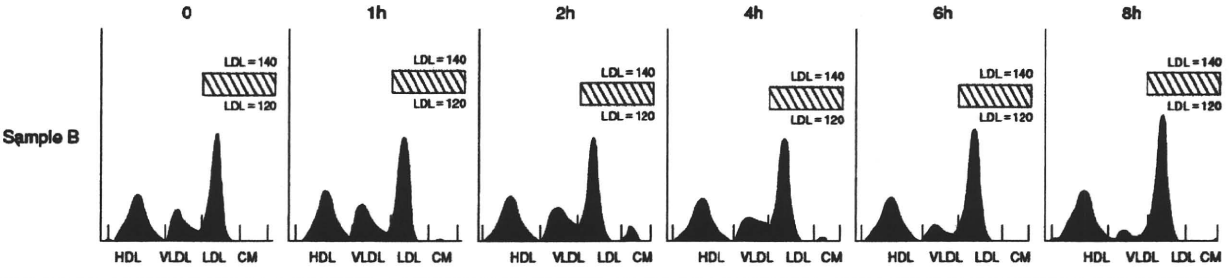
Discussion

In this study we analyzed serum lipids and apolipoproteins in fasting and postprandial periods among 24 young normolipidemic subjects. When we divided subjects into two groups according to the peak values of TG after loading, we found that the high TG group showed higher concentrations of TG, RemL-C, RLP-C, RLP-TG, and apoB-48 in the fasting period, although some parameters did not show significant differences. These concentrations were obviously elevated after loading in the high TG groups.

It is clear that an increase in chylomicrons and their remnants derived from the intestine is observed in the postprandial period; however, some investigators have reported that VLDL and their remnants derived from the liver are also increased, and it is



	0	1h	2h	4h	6h	8h
TC (mg/dL)	188	185	183	181	180	182
LDL-C (mg/dL)	124	118	113	111	115	117
HDL-C (mg/dL)	46	45	44	42	43	46
TG (mg/dL)	89	169	230	152	85	67



	0	1h	2h	4h	6h	8h
TC (mg/dL)	178	174	173	175	179	196
LDL-C (mg/dL)	92	88	88	94	91	101
HDL-C (mg/dL)	72	68	67	72	71	78
TG (mg/dL)	77	86	102	79	56	47

Fig. 2. Densitometric scanning electrophoretogram patterns of samples before and after loading the test meal. Elution profiles were analyzed by an automatic densitometer, Chol/Trig Combo™.

Sample A: Representative elution profile in the group with >150 mg/dL triglyceride (TG) concentration elevation after loading the test meal. Sample B: Representative elution profile in the group with <150 mg/dL TG concentration elevation before and after loading the test meal. HDL: high-density lipoproteins, VLDL: very-low-density lipoproteins, LDL: low-density lipoproteins, CM: chylomicrons, TG: triglyceride, red area: cholesterol, blue-colored area: TG.

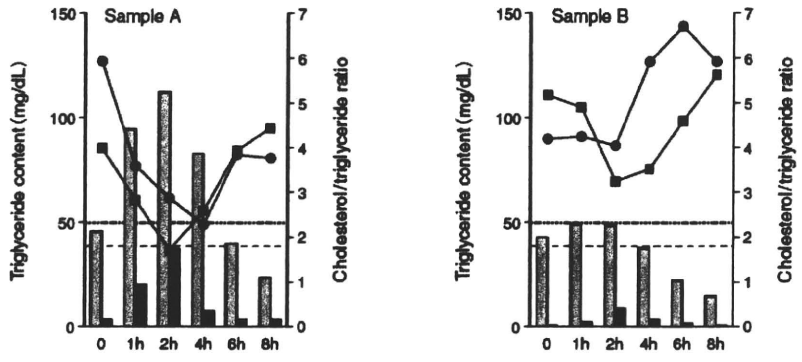


Fig. 3.

Changes in triglyceride (TG) levels of very-low-density lipoproteins (VLDL) and chylomicrons, and the ratios of cholesterol to TG of high-density lipoproteins (HDL) and low-density lipoproteins (LDL) before and after loading the test meal. Open column: TG content of VLDL, closed column: TG content of chylomicrons, closed circle: ratios of cholesterol to TG of HDL, closed square: ratio of cholesterol to TG of LDL, dashed line: ratio of cholesterol to TG of HDL of the mean-2SD in control subjects, dotted line: ratio of cholesterol to TG of LDL of the mean-2SD in control subjects.

controversial which is predominant in exogenous and endogenous lipids in the postprandial period[6, 22, 23]. Although the detailed mechanism remains unknown, the postprandial increase in VLDL and their remnants may be caused by reduced clearance, which was a result of competition by chylomicrons for the removal of triglycerides by lipoprotein lipase, or increased hepatic secretion of VLDL[22]. Receptor-mediated mechanisms are the predominant pathway by which chylomicron remnants are taken up by hepatocytes, and the LDL receptor pathway is thought to be the major mechanism for the uptake of both remnants with apoE as a ligand[12, 24, 25]. Impairment of or competition for the removal of both remnants in the liver may also potentially induce an increased retention time of these lipoproteins in the blood circulation. Thus, both remnant concentrations may be elevated in the postprandial period. In this study there was a remarkable elevation of apoB-48, which is a component of chylomicrons and chylomicron remnants, corroborating that lipoproteins derived from the intestine increase in the postprandial period, especially from 1 to 4 hours. ApoB concentrations (most derived from the liver) did not alter remarkably, suggesting that the postprandial increase in VLDL and their remnants may be small in young normolipidemic subjects.

Data of sample A (high TG group) demonstrated that delayed clearance of TG-rich lipoproteins in the postprandial period may be detected even in normolipidemic subjects. Impaired removal of TG-rich lipoproteins may induce the change of cholesterol and TG composition in LDL and HDL via the mechanism by which cholesteryl ester transfer protein is mediated[26, 27]; the higher the TG concentrations of chylomicrons and VLDL, the lower the ratios of cholesterol to TG of HDL and LDL. Thus, our data suggest that it may be possible to characterize lipid profiles conveniently in the fasting period by measuring TG, RemL-C, RLP-C, RLP-TG, and apoB-48 concentrations to detect postprandial hyperlipidemic subjects.

In the postprandial period, the elevation of RemL-C concentration did not reach a significant level, although the elevation period of RemL-C is similar to those of TG, RLP-C, and RLP-TG. These results may be due to the different measuring methods. RLP-C and RLP-TG were measured by the immune adsorption method[17], while RemL-C is measured by a newly developed and convenient assay for remnant lipoproteins[18]. This assay utilizes surfactant and phospholipase-D to directly solubilize and degrade remnants. As such, it can be performed with an automated clinical analyzer in a short time[18]. There was reportedly a strong correlation between RemL-C and RLP-C concentrations in patients with coronary artery disease[28]; however, our results (**Table 1**) suggest that differences in sensitivity for exogenous and endogenous lipoproteins between both methods may exist. The method for RLP-C and RLP-TG may be more sensitive to exogenous remnants, while RemL-C may be suitable for endogenous remnants. This hypothesis is compatible with previous reports[18, 28]. When we compared the two groups, the different character between RLP-C and RemL-C became clear (**Table 2** and **Fig. 1**). The high TG group had significantly higher fasting TG and RemL-C concentrations. After loading, RLP-C, RLP-TG, and apoB-48 also became significant parameters. It can be deduced that, in the fasting period, exogenous remnants in postprandial hyperlipidemic subjects may decrease to a similar level to that in normal subjects, and endogeneous remnants may remain at a significantly higher level.

There are some limitations of the present study as follows: as the study was performed with a small number of normal young subjects, only 4 individuals had the peak value of TG over 150 mg/dL as the high group. We need further examination with a larger number of normal and hyperlipidemic subjects in order to verify the TG value of 150 mg/dL, to identify the most dangerous lipid profile(s) involving remnants, apoB-48, and other parameters, and to clarify the significant differences between RLP-C and RemL-C. In addition, analyses of apoCs in remnant lipoproteins and LPL are necessary because these enzyme and proteins interfere with the apoE-mediated uptake of remnants and lipolysis[29, 30]. Here, since we did not examine LPL activity and protein mass measurement, we could not determine whether subjects in the high TG group have heterozygous LPL deficiency[31]. Of note, fasting sd-LDL-C concentration was higher in the high TG group. Interestingly, sd-LDL-C concentrations gradually decreased after loading. Here, we have no data to explain why sd-LDL-C in the high TG group but not in the low TG group gradually decreased after loading. Recently, Ogita *et al.* have reported that serum sd-LDL-C concentrations decreased after the 75 g oral glucose tolerance test and suggested that insulin can be a key modulator of sd-LDL-C concentrations[32].

In conclusion, TG, RLP-C, RLP-TG, RemL-C, and apoB-48 concentrations in the fasting period may be suitable to detect and characterize postprandial hyperlipidemia in normolipidemic subjects. In future, it is necessary to reveal which parameter or combination is useful to identify postprandial hyperlipidemia with a large-scale study.

References

1) Zilversmit DB: Atherogenesis: a postprandial phenomenon. Circulation, 1979; 60:473-485
2) Groot PH, van Stiphout WA, Krauss XH, Jansen H, van Tol A, van Ramshorst E, Chin-On S, Hofman A, Cresswell SR, Havekes L: Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. Arterioscler Thromb, 1991; 11:653-662
3) Patsch JR, Miesenböck G, Hopferwieser T, Mühlberger V, Knapp E, Dunn JK, Gotto AM. Jr, Patsch W: Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb, 1992; 12:1336-1345
4) Karpe F, Hamsten A: Postprandial lipoprotein metabolism and atherosclerosis. Curr Opin Lipidol, 1995; 6:123-129
5) Davignon J, Cohn JS: Triglycerides: a risk factor for coronary heart disease. Atherosclerosis, 1996; 124(Suppl):S57-S64
6) Yu KC, Cooper AD: Postprandial lipoproteins and atherosclerosis. Front Biosci, 2001; 6:D332-D354
7) Kugiyama K, Doi H, Takazoe K, Kawano H, Soejima H, Mizuno Y, Tsunoda R, Sakamoto T, Nakano T, Nakajima K, Ogawa H, Sugiyama S, Yoshimura M, Yasue H: Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. Circulation; 1999; 99:2858-2860
8) Nakamura T, Takano H, Umetani K, Kawabata K, Obata JE, Kitta Y, Kodama Y, Mende A, Ichigi Y, Fujioka D, Saito Y, Kugiyama K: Remnant lipoproteinemia is a risk factor for endothelial vasomotor dysfunction and coronary artery disease in metabolic syndrome. Atherosclerosis, 2005; 181:321-327
9) Twickler TB, Dallinga-Thie GM, Cohn JS, Chapman MJ: Elevated remnant-like particle cholesterol concentration: a characteristic feature of the atherogenic lipoprotein phenotype. Circulation, 2004; 109:1918-1925
10) Redgrave TG: Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. J Clin Invest, 1970; 49:465-471
11) Imaizumi K, Fainaru M, Havel RJ: Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. J Lipid Res, 1978; 19:712-722
12) Cooper AD: Hepatic uptake of chylomicron remnants. J Lipid Res, 1997; 38:2173-2192
13) Mahley RW, Ji ZS: Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. J Lipid Res, 1999; 40:1-16
14) Sakai N, Uchida Y, Ohashi K, Hibuse T, Saika Y, Tomari Y, Kihara S, Hiraoka H, Nakamura T, Ito S, Yamashita S, Matsuzawa Y: Measurement of fasting serum apo B-48 levels in normolipidemic and hyperlipidemic subjects by ELISA. J Lipid Res, 2003; 44:1256-1262
15) 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, Shirahashi N, Kita T: Prevalence of metabolic syndrome in the general Japanese population in 2000. J Atheroscler Thromb, 2006; 13:202-208
16) Hirano T, Ito Y, Yoshino G: Measurement of small dense low-density lipoprotein particles. J Atheroscler Thromb, 2005; 12:67-72
17) Nakajima K, Saito T, Tamura A, Suzuki M, Nakano T, Adachi M, Tanaka A, Tada N, Nakamura H, Campos E, Havel RJ: Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels. Clin Chim Acta, 1993; 223:53-71
18) Miyauchi K, Kayahara N, Ishigami M, Kuwata H, Mori H, Sugiuchi H, Irie T, Tanaka A, Yamashita S, Yamamura T: Development of a homogeneous assay to measure remnant lipoprotein cholesterol. Clin Chem, 2007; 53:2128-2135
19) Sato I, Taniguchi T, Ishikawa Y, Kusuki M, Hayashi F, Mukai M, Kawano S, Kondo S, Yamashita S, Kumagai S: The lipoprotein fraction between VLDL and LDL detected by biphasic agarose gel electrophoresis reflects serum remnant lipoprotein and Lp(a) concentrations. J Atheroscler Thromb, 2006; 13:55-61
20) Kido T, Kurata H, Matsumoto A, Tobiyama R, Musha T, Hayashi K, Tamai S, Utsunomiya K, Tajima N, Fidge N, Itakura H, Kondo K: Lipoprotein analysis using agarose gel electrophoresis and differential staining of lipids. J Atheroscler Thromb, 2001; 8:7-13
21) Sato I, Hyakuta M, Hayashi F, Mukai M, Kondo S, Maeda E, Kumagai S: Usefulness of examination of the cholesterol versus triglyceride ratio for lipoprotein fractions in a patient with marked hyper-triglyceridemia. Rinsho Byori, 2002; 50:987-991
22) Karpe F: Postprandial lipoprotein metabolism and atherosclerosis. J Intern Med, 1999; 246:341-355
23) Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN: Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. J Lipid Res, 2003; 44:2065-2272
24) Mahley RW: Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science, 1988; 240:622-630
25) 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
26) Yamashita S, Sakai N, Hirano K, Ishigami M, Maruyama T, Nakajima N, Matsuzawa Y: Roles of plasma lipid transfer proteins in reverse cholesterol transport. Front Biosci, 2001; 6:D366-D387
27) Taskinen MR, Smith U: Lipid disorders in NIDDM: implications for treatment. J Intern Med, 1998; 244:361-370
28) Nakada Y, Kurosawa H, Tohyama J, Inoue Y, Ikewaki K: Increased remnant lipoprotein in patients with coronary artery disease--evaluation utilizing a newly developed remnant assay, remnant lipoproteins cholesterol homogenous assay (RemL-C). J Atheroscler Thromb, 2007; 14:56-64
29) Shachter NS: Apolipoproteins C-I and C-III as important modulators of lipoprotein metabolism. Curr Opin Lipidol, 2001; 12:297-304
30) Westerterp M, de Haan W, Berbée JF, Havekes LM, Rensen PC: Endogenous apoC-I increases hyperlipidemia in apoE-knockout mice by stimulating VLDL production and inhibiting LPL. J Lipid Res, 2006; 47:1203-1211

31) Fojo SS, Brewer HB: Hypertriglyceridaemia due to genetic defects in lipoprotein lipase and apolipoprotein C-II. J Intern Med, 1992; 231:669-677
32) Ogita K, Ai M, Tanaka A, Ito Y, Hirano T, Yoshino G, Shimokado K: Serum concentration of small dense low-density lipoprotein-cholesterol during oral glucose tolerance test and oral fat tolerance test. Clin Chem Acta, 2008; 387:36-41

Clinica Chimica Acta 410 (2009) 31–38

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Differential reactivities of four homogeneous assays for LDL-cholesterol in serum to intermediate-density lipoproteins and small dense LDL: Comparisons with the Friedewald equation

Shizuya Yamashita [a,*], Ryota Kawase [a], Hajime Nakaoka [a], Kazuhiro Nakatani [a], Miwako Inagaki [a], Miyako Yuasa-Kawase [a], Kazumi Tsubakio-Yamamoto [a], Jose C. Sandoval [a], Daisaku Masuda [a], Tohru Ohama [a], Yumiko Nakagawa-Toyama [a,b], Akifumi Matsuyama [c], Makoto Nishida [a,b], Masato Ishigami [a,d]

[a] Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Health Care Center, Osaka University, Osaka, Japan
[c] Laboratory for Somatic Stem Cell Therapy, Foundation of Biomedical Research and Innovation, Kobe, Japan
[d] Department of Biomedical Informatics, Division of Health Sciences, Osaka University Graduate School of Medicine, Osaka, Japan

ARTICLE INFO

Article history:
Received 26 January 2009
Received in revised form 4 September 2009
Accepted 4 September 2009
Available online 12 September 2009

Keywords:
LDL-cholesterol
Homogeneous assay
Type III hyperlipoproteinemia
Intermediate-density lipoproteins
Small dense LDL

ABSTRACT

Background: In routine clinical laboratory testing and numerous epidemiological studies, LDL-cholesterol (LDL-C) has been estimated commonly using the Friedewald equation. We investigated the relationship between the Friedewald equation and 4 homogeneous assays for LDL-C.
Methods: LDL-C was determined by 4 homogeneous assays [liquid selective detergent method: LDL-C (L), selective solubilization method: LDL-C (S), elimination method: LDL-C (E), and enzyme selective protecting method: LDL-C (P)]. Samples with discrepancies between the Friedewald equation and the 4 homogeneous assays for LDL-C were subjected to polyacrylamide gel electrophoresis and the β-quantification method.
Results: The correlations between the Friedewald equation and the 4 homogeneous LDL-C assays were as follows: LDL-C (L) ($r=0.962$), LDL-C (S) ($r=0.986$), LDL-C (E) ($r=0.946$) and LDL-C (P) ($r=0.963$). Discrepancies were observed in sera from type III hyperlipoproteinemia patients and in sera containing large amounts of midband and small dense LDL on polyacrylamide gel electrophoresis. LDL-C (S) was most strongly correlated with the β-quantification method even in sera from patients with type III hyperlipoproteinemia.
Conclusions: Of the 4 homogeneous assays for LDL-C, LDL-C (S) exhibited the closest correlation with the Friedewald equation and the β-quantification method, thus reflecting the current clinical databases for coronary heart disease.

1. Introduction

Numerous clinical studies have shown an independent relationship between increases in LDL-cholesterol (LDL-C) concentrations and risk of coronary heart disease (CHD) [1,2]. According to the National Cholesterol Education Program (NCEP)-Adult Treatment Panel III, the diagnosis and management of adult patients with hypercholesterolemia is largely based on the concentration of LDL-C [3].

A wide variety of methods have been used for determining LDL-C in serum. These methods include sequential and density-gradient ultracentrifugation [4], β-quantification [5–8], Friedewald equation [9], electrophoresis [10], HPLC [11] and homogeneous assay [12]. As a reference procedure for LDL-C, the CDC has adopted a variation of the multi-step β-quantification procedure used by the Lipid Research Clinics [13], which combines separation by ultracentrifugation and chemical precipitation. The β-quantification procedure for LDL-C has been also recommended by the NCEP Lipoprotein Measurement Working Group [7]. However, the β-quantification method requires a relatively large volume of serum, special equipment, and is a time-consuming procedure; therefore, it is not well suited for routine testing in hospitals and clinics. Although the Friedewald equation is the most commonly used technique in clinical laboratories for the estimation of LDL-C, it cannot be accurately estimated when plasma triglycerides (TG) >4.52 mmol/l (400 mg/dl) or when specimens are

Abbreviations: LDL-C, LDL-cholesterol; CHD, coronary heart disease; NCEP, National Cholesterol Education Program; TG, triglycerides; IDL, intermediate-density lipoproteins; apo, apolipoprotein; CRMLN, Cholesterol Reference Method Laboratory Network; PAGE, polyacrylamide gel electrophoresis; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C (F), LDL-C estimated by Friedewald equation; LDL-C (L), liquid selective detergent method; LDL-C (S), selective solubilization method; LDL-C (E), elimination method; LDL-C (P), enzyme selective protecting method; LDL-C (BQ), modified β-quantification method; LDL-MI, LDL migration index.

* Corresponding author. Tel.: +81 6 6879 3732; fax: +81 6 6879 3739.
E-mail address: shizu@imed2.med.osaka-u.ac.jp (S. Yamashita).

0009-8981/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2009.09.010

collected in the non-fasting state [5,14]. In most clinical studies investigating the relationship between LDL-C and CHD, including the Framingham Heart Study and the study of the prediction of CHD, LDL-C was estimated by the Friedewald equation [15–17]. In the NCEP-Adult Treatment Panel III study, the recommended level of LDL-C for the prevention of CHD was also estimated by the Friedewald equation. Like the β-quantification method, LDL-C estimated by the Friedewald equation includes cholesterols in lipoprotein (a) and intermediate-density lipoproteins (IDL) [5,7,18], which are atherogenic apolipoproptein (apo) B-containing lipoproteins, and there is a close correlation between the β-quantification method and the Friedewald equation [5,19].

In recent years, convenient homogeneous assays for LDL-C that are less influenced by TG have been developed and are widely performed at clinical laboratories. As mentioned above, LDL-C, which has been shown to be a risk factor for CHD by clinical studies, is estimated by the Friedewald equation, and some studies have stated that LDL-C measured by homogeneous assays for LDL-C need to reflect the current clinical databases for CHD risks as assessed by the Friedewald equation [7]. Clinicians have also expressed the need for a homogeneous assay for LDL-C reflecting LDL-C as estimated by the Friedewald equation [7]. Most homogeneous assays for LDL-C have received CDC certification from the Cholesterol Reference Method Laboratory Network (CRMLN) [12]. Because different homogeneous assays for LDL-C employ different measurement principles, discrepancies have been reported with samples having IDL, lipoprotein (a) and abnormal lipoproteins associated with liver dysfunction, such as lipoprotein X or lipoprotein Y [20–22].

We investigated the correlations between the Friedewald equation and 4 homogeneous assays developed in Japan and to determine whether the homogeneous assays for LDL-C reflect the clinical databases for CHD where LDL-C was estimated by the Friedewald equation. Samples with discrepancies between the Friedewald equation and homogeneous assays for LDL-C were analyzed by polyacrylamide gel electrophoresis (PAGE) and the β-quantification method.

2. Materials and methods

2.1. Samples

With approval from the Ethics Review Board of Osaka University Hospital, human serum samples were from 156 patients (age: 30–65 years) with <4.52 mmol/l (400 mg/dl) TG and from 5 patients (age: 58–75 years) with type 2 diabetes mellitus. Informed consent was obtained from all patients. After 12 h of fasting, blood samples were collected in tubes without anticoagulant. Blood samples were stored at 4 °C and were measured within 3 days using a Hitachi 7170 analyzer.

2.2. Procedures

2.2.1. Total cholesterol (TC), TG and HDL-cholesterol (HDL-C) assays

TC and TG were measured by an enzymatic method (Kyowa Medex) according to the manufacturer's protocols. The CVs for TC and TG were <1.5 and 2%, respectively. HDL-C was measured by a modified enzymatic method (Kyowa Medex) [12] according to the manufacturer's protocols. The CV for HDL-C was <1.5%.

2.2.2. Friedewald equation [9]

Using samples with <4.52 mmol/l (400 mg/dl) TG, LDL-C in the Friedewald equation was estimated using the following formula: [LDL-C (F)] = [TC] − [HDL-C] − [TG]/2.22. HDL-C concentrations in the Friedewald equation were measured by a modified enzymatic method (Kyowa Medex) [12].

Table 1
Lipid levels, PAGE[a] and apoE phenotype of discrepancy samples and samples from diabetic patients.

Sample	Total cholesterol	Triglycerides	PAGE		apo E phenotype
	mmol/l (mg/dl)	mmol/l (mg/dl)	midband[b]	LDL-MI[c]	
Discrepancy samples					
A	4.43 (171)	1.11 (99)	+	0.24	E2/2
B	4.76 (184)	1.42 (127)	+	0.23	E2/2
C	4.60 (178)	0.93 (83)	+	0.31	E2/2
D	6.11 (236)	1.86 (166)	+	0.36	E2/2
E	4.74 (183)	1.36 (121)	+	0.29	E2/2
F	4.45 (172)	1.04 (93)	+	0.31	E2/2
G	5.27 (204)	3.40 (303)	+	0.41	E2/2
H	4.76 (184)	3.38 (302)	+	0.48	E3/3
I	7.86 (304)	4.40 (393)	+	0.40	E3/3
Diabetic patients					
J	5.63 (218)	2.03 (181)	+	0.38	E3/3
K	5.27 (204)	4.18 (373)	+	0.41	E3/2
L	6.92 (268)	6.38 (569)	+	0.44	E3/2
M	4.65 (180)	3.06 (273)	+	0.41	E3/3
N	5.77 (223)	1.36 (121)	+	0.43	E3/3

[a] PAGE, polyacrylamide gel electrophoresis.
[b] When an independent peak was observed between the β and pre-β lipoprotein bands or when a shoulder was observed on the pre-β side of the β lipoprotein, the midband was interpreted as positive.
[c] LDL-MI, LDL migration index. The LDL-MI was obtained by dividing the distance from the LDL peak to VLDL peak (LVp) by the distance from the HDL peak to VLDL peak (HVp), and when LVp/HVp was ≥0.40, the existence of small dense LDL is inferred.

2.2.3. LDL-C homogeneous assays

LDL-C was determined by 4 homogeneous assays [liquid selective detergent method (Sekisui Medical): LDL-C (L), selective solubilization method (Kyowa Medex): LDL-C (S), elimination method (Denka Seiken): LDL-C (E), and enzyme selective protecting method (Wako Pure Chemical): LDL-C (P)] [12]. LDL-C was measured according to the manufacturers' protocols. The CV was <2% for LDL-C (S), <2% for LDL-C (L), <2% for LDL-C (E), and <2% for LDL-C (P).

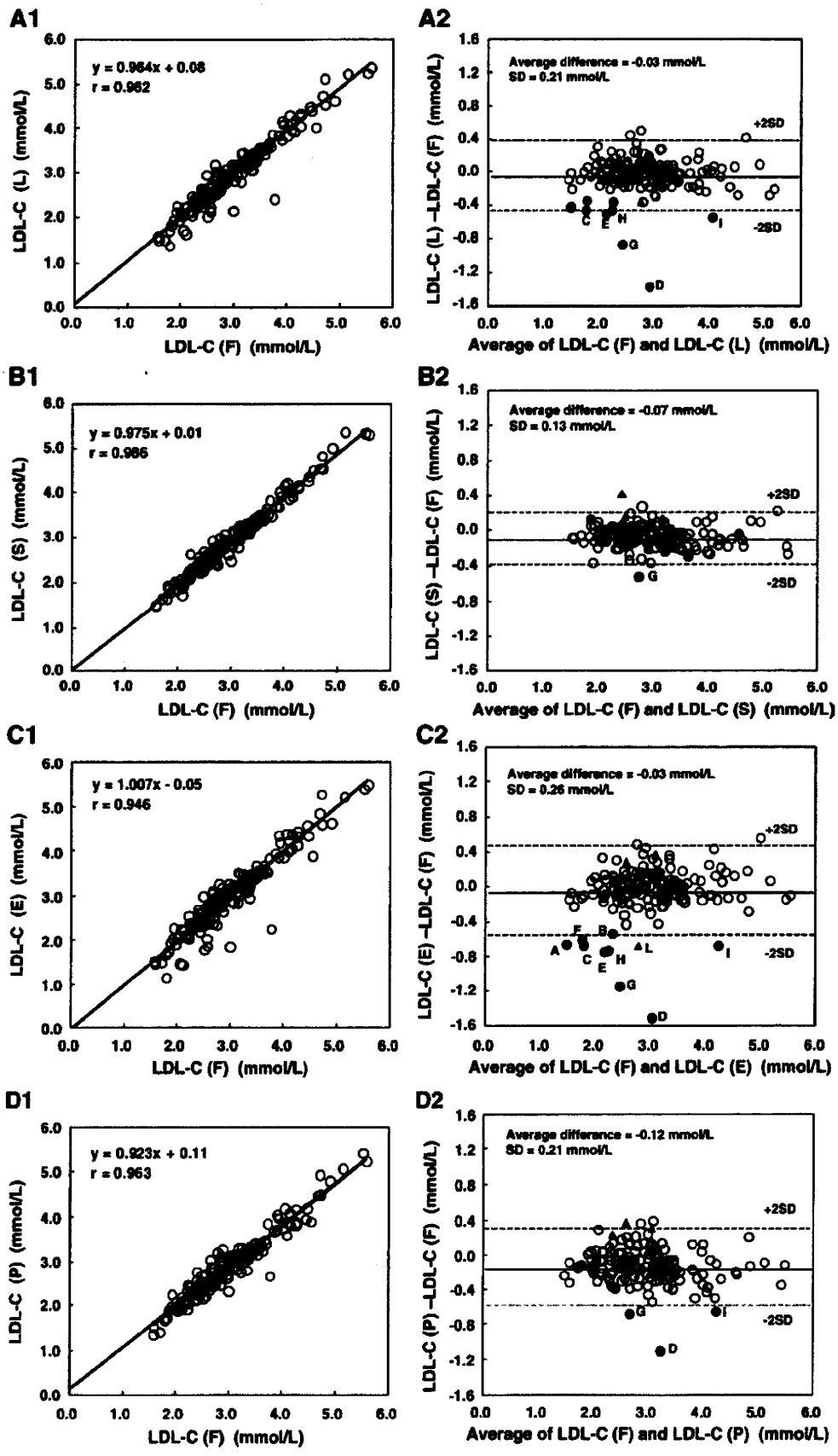
2.2.4. Modified β-quantification method [LDL-C (BQ)]

We employed the method described in the Handbook of Lipoprotein Testing, 2nd ed. [5,6]. After ultracentrifugation at a density of 1.006 kg/l with 0.8 ml serum, the bottom fraction was obtained by the heparin-Mn^{2+} precipitation method, and the TC for the bottom fraction and after fractionation was measured by the enzymatic method (Kyowa Medex). To check the accuracy of the LDL-C (BQ), the values obtained by LDL-C (BQ) were compared with those of the β-quantification method at the CRMLN Laboratory of Osaka Medical Center for Health Science and Promotion in Osaka, Japan, which uses a heparin-Mn^{2+} precipitation with subsequent cholesterol determination by Abell–Kendall method [6], in fasting serum samples from 11 healthy volunteers. LDL-C (BQ) was found to be highly correlated with the β-quantification method of CRMLN (see Supplemental data Table 1).

2.2.5. PAGE and apoE phenotyping

We performed PAGE using Lipophor system (Jokoh) according to the manufacturer's instructions. The lipoprotein bands were

Fig. 1. Correlations between the Friedewald equation and the 4 homogeneous assays for LDL-C. Both the linear regression and the Bland–Altman difference plot are shown. *(A)*, LDL-C (F) vs. LDL-C (L). *(B)*, LDL-C (F) vs. LDL-C (S). *(C)*, LDL-C (F) vs. LDL-C (E). *(D)*, LDL-C (F) vs. LDL-C (P). Fresh fasting serum samples ($n = 160$) including samples from diabetic patients with <4.52 mmol/l TG were used. The closed circles indicate samples being smaller than the lower limit of 2 SD of LDL-C (E), which showed the largest SD among the homogenous assays for LDL-C. The closed triangles indicate samples from diabetic patients.


A1
y = 0.964x + 0.08
r = 0.962
LDL-C (L) (mmol/L)
LDL-C (F) (mmol/L)
A2
Average difference = -0.03 mmol/L
SD = 0.21 mmol/L
+2SD
-2SD
LDL-C (L) –LDL-C (F) (mmol/L)
Average of LDL-C (F) and LDL-C (L) (mmol/L)
B1
y = 0.975x + 0.01
r = 0.986
LDL-C (S) (mmol/L)
LDL-C (F) (mmol/L)
B2
Average difference = -0.07 mmol/L
SD = 0.13 mmol/L
+2SD
-2SD
LDL-C (S) –LDL-C (F) (mmol/L)
Average of LDL-C (F) and LDL-C (S) (mmol/L)
C1
y = 1.007x - 0.05
r = 0.946
LDL-C (E) (mmol/L)
LDL-C (F) (mmol/L)
C2
Average difference = -0.03 mmol/L
SD = 0.26 mmol/L
+2SD
-2SD
LDL-C (E) –LDL-C (F) (mmol/L)
Average of LDL-C (F) and LDL-C (E) (mmol/L)
D1
y = 0.923x + 0.11
r = 0.963
LDL-C (P) (mmol/L)
LDL-C (F) (mmol/L)
D2
Average difference = -0.12 mmol/L
SD = 0.21 mmol/L
+2SD
-2SD
LDL-C (P) –LDL-C (F) (mmol/L)
Average of LDL-C (F) and LDL-C (P) (mmol/L)

densitometrically determined. When an independent peak was observed between the β and pre-β lipoprotein bands or when a shoulder was observed on the pre-β side of the β lipoprotein, the midband was interpreted as positive [23]. LDL particle size was also simply estimated based on an LDL migration index (LDL-MI) [24,25]. The LDL-MI was obtained by dividing the distance from the LDL peak to VLDL peak (LVp) by the distance from the HDL peak to VLDL peak (HVp), and when LVp/HVp was ≥0.40, the existence of small dense LDL is inferred. We identified the apoE phenotype of samples with a Phenotyping ApoE Kit (Jokoh) using the immunoblotting method.

2.2.6. Statistics

Pearson correlation coefficient analysis, simple regression, the Bland–Altman plot [26] and the Boxplot were used to assess the relation between the Friedewald equation, the β-quantification, and the 4 homogenous assays for LDL-C. *P* values were obtained from the Kruskal–Wallis test followed by Dunn's multiple comparison test. Statistical analysis was performed using Excel 2003 (Microsoft) with the Statcel 2 software plug-in [27] or GraphPad Prism software with Windows XP.

3. Results

3.1. Comparison between the Friedewald equation and homogeneous assays for LDL-C

The 4 homogeneous assays for LDL-C were compared with the Friedewald equation in fasting serum samples from 156 patients and 4 diabetic patients with <4.52 mmol/l TG. As shown in Fig. 1, LDL-C (S) was correlated most closely with LDL-C (F), with a correlation coefficient of 0.986 ($P<0.001$), and a regression line of $y=0.975x+0.01$ mmol/l, the extent of agreement decreasing in the order of LDL-C (P) ($r=0.963$, $P<0.001$, $y=0.923x+0.11$ mmol/l) > LDL-C (L) ($r=0.962$, $P<0.001$, $y=0.964x+0.08$ mmol/l) > LDL-C (E) ($r=0.946$, $P<0.001$, $y=1.007x-0.05$ mmol/l). Fig. 1 also shows the Bland–Altman bias plot of the comparison between LDL-C (F) and the 4 homogeneous assays for LDL-C. The SD were 0.21 mmol/l (8.2 mg/dl) for LDL-C (L), 0.13 mmol/l (4.9 mg/dl) for LDL-C (S), 0.26 mmol/l (10.2 mg/dl) for LDL-C (E) and 0.21 mmol/l (7.9 mg/dl) for LDL-C (P). The average differences (*y*-axis) were −0.03 mmol/l (−1.2 mg/dl) for LDL-C (L), −0.07 mmol/l (−2.7 mg/dl) for LDL-C (S), −0.03 mmol/l (−1.0 mg/dl) for LDL-C (E) and −0.12 mmol/l (−4.7 mg/dl) for LDL-C (P). The difference between the Friedewald equation and the homogeneous assays for LDL-C (S) and LDL-C (P) was significantly smaller than that for LDL-C (L) and LDL-C (E) (Fig. 2). It should be noted that the homogenous assays for LDL-C occasionally exhibited lower concentrations of LDL-C than the Friedewald equation. The closed circles in Fig. 1 were the values that are smaller than the lower limit of 2 SD of LDL-C (E), which showed the largest SD among the homogenous assays for LDL-C.

3.2. Analysis by PAGE and identification of apoE phenotypes by immunoblotting of discrepancy samples

The 9 samples with discrepancies (samples A, B, C, D, E, F, G, H and I with closed circles in Fig. 1 and the 5 samples from diabetic patients (samples J, K, L, M and N with closed triangle in Fig. 1) were analyzed by PAGE (Fig. 3), and apoE phenotypes were identified by immunoblotting (Table 1). With samples A through G [TC: 4.43–6.11 mmol/l (171–236 mg/dl), TG: 0.93–3.40 mmol/l (83–303 mg/dl)], a midband was observed and the apoE phenotype in all the seven samples was E2/2, indicating type III hyperlipoproteinemia. With samples G through I [TC: 4.76–7.86 mmol/l (184–304 mg/dl), TG: 3.38–4.40 mmol/l (302–393 mg/dl)], PAGE showed a midband, and their LDL-MI values were ≥0.40, which indicate the existence of small dense LDL [24,25]. With samples J through N [TC: 4.65–6.92 mmol/l (180–268 mg/dl), TG: 1.36–6.38 mmol/l (121–569 mg/dl)], PAGE showed a midband, and the LDL-MI values of samples K through N were ≥0.40. The apoE phenotypes of samples J through N were E3/3 or E3/2.

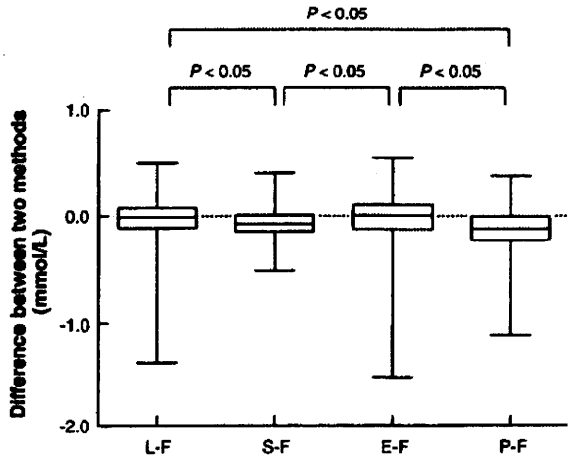


Fig. 2. Boxplots of the difference between the homogeneous assays and the Friedewald equation. Values are represented as median ± interquartile range with upper and lower limits. L−F, the difference between LDL-C (L) and LDL-C (F). S−F, the difference between LDL-C (S) and LDL-C (F). E−F, the difference between LDL-C (E) and LDL-C (F). P−F, the difference between LDL-C (P) and LDL-C (F).

3.3. Analysis by LDL-C (BQ) of discrepancy samples and samples from diabetic patients

The 7 type III hyperlipoproteinemia samples (samples A through G) and the five samples from diabetic patients (samples J through N) were analyzed by LDL-C (BQ). Fig. 4 shows the Bland–Altman bias plot of the comparison between LDL-C (BQ) and 4 homogeneous assays for LDL-C. Judging by the average difference and SD, LDL-C (S) [average difference 0.08 mmol/l (3.1 mg/dl), SD 0.18 mmol/l (7.0 mg/dl)] mostly agreed with LDL-C (BQ) as well as LDL-C (F) [average difference 0.06 mmol/l (2.3 mg/dl), SD 0.23 mmol/l (9.0 mg/dl)], the extent of agreement decreasing in the order of LDL-C (P) [average difference −0.06 mmol/l (−2.5 mg/dl), SD 0.28 mmol/l (10.9 mg/dl)] > LDL-C (L) [average difference −0.27 mmol/l (−10.4 mg/dl), SD 0.33 mmol/l (12.8 mg/dl)] > LDL-C (E) [average difference −0.40 mmol/l (−15.4 mg/dl), SD 0.48 mmol/l (18.5 mg/dl)]. Of the 5 methods tested, the difference from the β-quantification method for LDL-C (S) and the Friedewald equation was significantly smaller than that for LDL-C (E) (Fig. 5). It should be noted that downward deviations in LDL-C (L) and LDL-C (E) against LDL-C (BQ) were observed for the samples from the patients with type III hyperlipoproteinemia, unlike those from the diabetic patients (Fig. 4).

4. Discussion

In most clinical studies investigating the relationship between LDL-C and CHD, LDL-C was estimated by the Friedewald equation or the β-quantification method [15–17]. Although LDL-C is generally considered to be a major risk factor for progression of atherosclerosis, the traditional measurement of LDL-C includes measurement of IDL [18]. Like the β-quantification method, LDL-C estimated by the Friedewald equation includes cholesterols in lipoprotein (a) and IDL [5,7,18], which are atherogenic apoB-containing lipoproteins. In recent years, homogeneous assays for LDL-C have been widely used, but when referring to the clinical databases for CHD risks, as assessed based on LDL-C estimated by the Friedewald equation or the β-quantification method, homogeneous assays for LDL-C should reflect

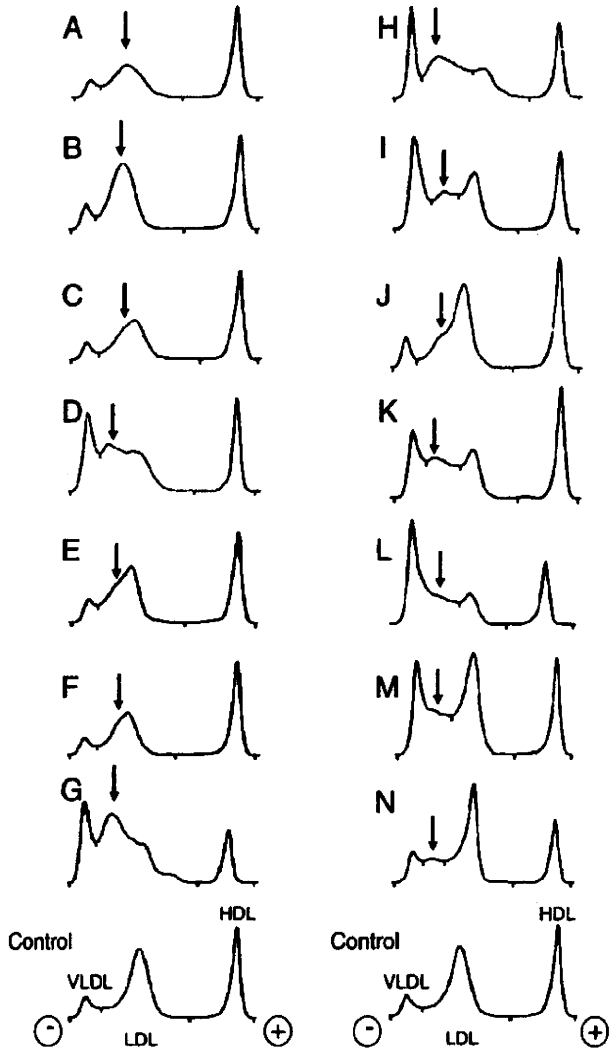


Fig. 3. Densitometric patterns of PAGE (polyacrylamide gel electrophoresis) of discrepancy samples and samples from diabetic patients. Control, normal serum. (A), sample A. (B), sample B. (C), sample C. (D), sample D. (E), sample E. (F), sample F. (G), sample G. (H), sample H. (I), sample I. (J), sample J. (K), sample K. (L), sample L. (M), sample M. (N), sample N.; downward closed arrows, location of midband.

the LDL-C measurements from the Friedewald equation or the β-quantification method [7]. We investigated the correlations between the Friedewald equation and 4 homogeneous assays developed in Japan to determine whether the homogeneous assays for LDL-C reflect the clinical databases for CHD where LDL-C was estimated by the Friedewald equation.

Using 156 fasting serum samples and 4 samples from diabetic patients with <4.52 mmol/l (400 mg/dl) TG, the correlations between the Friedewald equation and the 4 homogeneous assays for LDL-C were investigated because the Friedewald equation cannot be used to estimated LDL-C in samples with ≥4.52 mmol/l (400 mg/dl) TG [5,14]. Of the homogenous assays evaluated, LDL-C (S) was correlated most closely with LDL-C (F). However, the homogenous assays for LDL-C occasionally exhibited lower concentrations of LDL-C than the Friedewald equation (Fig. 1). When the samples showing lower concentrations were analyzed by PAGE, a large amount of midband was detected in samples A through G, indicating increased concentrations of IDL (Fig. 3) [23]. Furthermore, when these samples were analyzed by immunoblotting, apoE2/2 was identified (Table 1). It was suggested that samples A through G are from patients with type III hyperlipoproteinemia, indicating increased levels of IDL or β-VLDL [28,29]. When samples G through I were analyzed by PAGE, a midband was detected (Fig. 3), and their LDL-MI values were ≥0.40 (Table 1) [24,25]. This suggests that samples G through I contain a large volume of small dense LDL. When samples J through N from diabetic patients were analyzed by PAGE, a midband was detected (Fig. 3), and the LDL-MI values of sample K through N were ≥0.40 (Table 1), suggesting that the samples contain IDL and small dense LDL.

LDL-C (F) and the 4 homogeneous assays for LDL-C were compared with LDL-C (BQ), using 7 samples from the patients with type III hyperlipoproteinemia (samples A through G) and 5 samples from diabetic patients (samples J through N). Of the method tested, LDL-C (S) and the Friedewald equation mostly agreed with LDL-C (BQ), whereas a consistent mean negative bias of LDL-C (E), LDL-C (L) and LDL-C (P) against LDL-C (BQ) was observed more apparent in the Bland–Altman plot (Fig. 4). In the samples, sample D exhibited the largest difference among the measurements methods. LDL-C (S) mostly agreed with LDL-C (BQ) in sample D, whereas LDL-C (F) exhibited slightly higher values and other LDL-C homogeneous measurements exhibited markedly lower values. In general, using type III hyperlipoproteinemia samples, upward deviations in the Friedewald equation against LDL-C (BQ) have been reported [5,14]. Nevertheless, in the present study, there was no significant difference between the 2 methods (Fig. 4). Based on the medical records of the patients (A to F) with type III hyperlipoproteinemia, these patients were managed with lipid-lowering drugs, such as fenofibrate or bezafibrate, and the serum concentrations of TC and TG in the patients were maintained at low levels. In such a situation, upward deviations in the Friedewald equation against the LDL-C (BQ) might not be able to be caused even in the patients with type III hyperlipoproteinemia.

As shown in Figs. 1 and 4, marked downward deviations were observed in LDL-C (L) and LDL-C (E) methods against the Friedewald equation and β-quantification method, especially in the samples from the patients with type III hyperlipoproteinemia, not in those from diabetic patients. The composition or amounts of IDL and triglyceride-rich lipoprotein remnants in the samples from the patients with type III hyperlipoproteinemia seem to differ from those from the diabetic patients. In fact, the cholesterol content and the cholesterol–triglycerides ratio in IDL from the patients with type III hyperlipoproteinemia are reported to be larger than those from the diabetic patients [30,31]. Such a difference seems to be reflected in the difference in the reactivities for the homogenous assays for LDL-C [17].

In this study, 4 homogeneous assays with different measurement principles were used for LDL-C [LDL-C (L), LDL-C (S), LDL-C (E), and LDL-C (P)] [12]. However, the measurement principles for the homogeneous assays for LDL-C can be divided into two groups: 1) method I in which cholesterols in lipoproteins, other than LDL, are eliminated in the first reaction and cholesterol in LDL is detected in the second reaction [LDL-C (L), LDL-C (E) and LDL-C (P)]; and 2) method II in which LDL is selectively modified by surfactants, making its component cholesterol available for enzymatic assay [LDL-C (S)]. In type III hyperlipoproteinemia, the results tended to be low for method I [LDL-C (L), LDL-C (E) and LDL-C (P)] (Fig. 4B, D and E). With the method I, cholesterols in HDL, VLDL and chylomicron are eliminated in the first reaction, and LDL-C is measured in the second reaction. Unlike LDL, IDL from the patients with type III hyperlipoproteinemia has not only apoB but also apoE and apoCs, which are relatively hydrophilic. Therefore, the surface structure of such lipoproteins differs from that of the LDL in terms of charge, while the apolipoprotein composition of such lipoproteins is similar to that of the VLDL [32]. In the case of samples with higher IDL levels from the patients with type III hyperlipoproteinemia, therefore, with the method I some portion of cholesterol in such IDL may also be eliminated

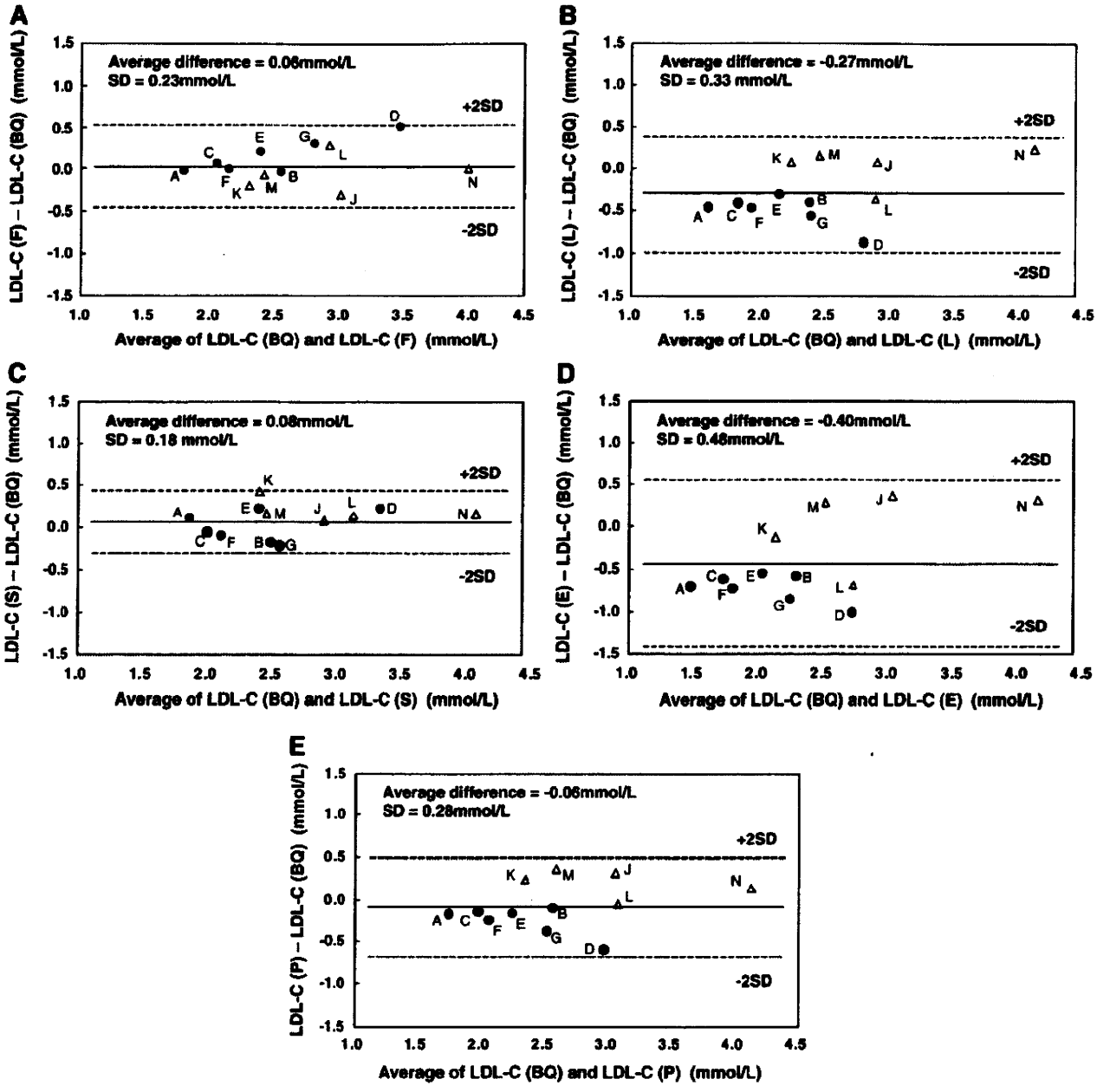


Fig. 4. Correlations between LDL-C (BQ), LDL-C (F) and 4 homogeneous assays for LDL-C in discrepancy samples and samples from diabetic patients. The Bland–Altman difference plot is shown. (A), LDL-C (BQ) vs. LDL-C (F). (B), LDL-C (BQ) vs. LDL-C (L). (C), LDL-C (BQ) vs. LDL-C (S). (D), LDL-C (BQ) vs. LDL-C. (E), LDL-C (BQ) vs. LDL-C (P). Seven type III hyperlipoproteinemia samples (samples A, B, C, D, E, F and G with closed circles) and five samples from diabetic patients (samples J, K, L, M and N with open triangles) were used.

during the first reaction. Accordingly, LDL-C measurements with the method I may lead to lower values than those of the β-quantification method and the Friedewald equation. With the method II, it has been reported that the nonionic surfactant, polyoxyethylene-polyoxypropylene block copolyether, may be able to recognize differences in hydrated density, net charge, or size of the various lipoprotein fractions [33]. It is most likely that such specific surfactant used in LDL-C (S) method shows the specificity toward IDL from the patients with type III hyperlipoproteinemia as well as LDL. On the other hand, Miller et al. [34] reported that the selective solubilization method for LDL-C measurement, corresponding to LDL-C (S) in this study, gave the big difference against the β-quantification method with the specimens from type III hyperlipoproteinemia. In this study, we used a currently commercially available reagent purchased from the manufacturer, in which the non-specific reactivity toward cholesterol in triglyceride-rich lipoprotein remnants from type III hyperlipoproteinemia are sure to be improved. It has been reported that IDL concentrations increase in serum samples from patients with type III hyperlipoproteinemia, diabetic mellitus [35], or myocardial infarction [23]. Furthermore, accumulating evidence indicates that IDL is associated with the presence, severity, and progression of coronary artery atherosclerosis [18,36,37]. In serum samples from the patients with increased IDL from type III hyperlipoproteinemia, the method I may

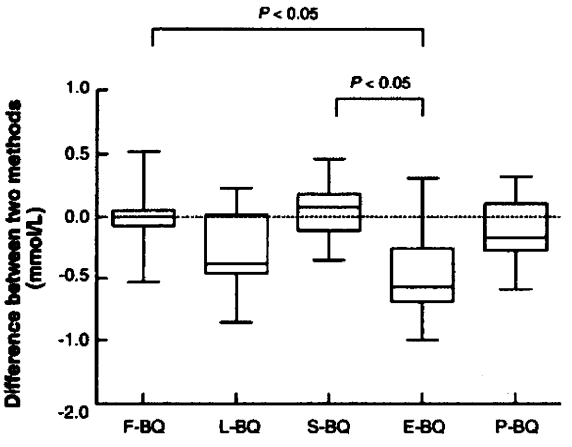


Fig. 5. Boxplots of the difference between LDL-C (BQ), LDL-C (F) and 4 homogeneous assays. Values are represented as median ± interquartile range with upper and lower limits. F − BQ, the difference between LDL-C (BQ) and LDL-C (F). L − BQ, the difference between LDL-C (L) and LDL-C (BQ). S − BQ, the difference between LDL-C (S) and LDL-C (BQ). E − BQ, the difference between LDL-C (E) and LDL-C (BQ). P − BQ, the difference between LDL-C (P) and LDL-C (BQ).

underestimate the LDL-C values compared with the Friedewald equation and the β-quantification method.

On the other hand, in this study, the significant differences among LDL-C methods have not been observed in serum samples from the diabetic patients with increased IDL. Further studies are needed to clarify such differential reactivity of IDL from type III hyperlipoproteinemia and diabetic patients in LDL-C homogeneous assays.

Using some samples with higher small dense LDL, the homogeneous assays for LDL-C exhibited lower values compared to LDL-C (F) (Fig. 1). Compared to normal LDL, small dense LDL has a different overall three-dimensional structure of apoB and immunoreactivity at the apoB receptor recognition domain [38]. In small dense LDL, being smaller than LDL, there is significant loss of phospholipids from the surface, loss of cholesterol esters from the core, gain of TG into the core, and most importantly, loss of free cholesterol from both the surface and the core [39]. Furthermore, compared to normal size LDL, small dense LDL has intrinsically increased susceptibility to LDL oxidation and aggregation [40,41]. As described above, small dense LDL is different from normal LDL in terms of structure and the property on lipoprotein. Therefore, unlike LDL, small dense LDL might not be fully recognized to the LDL-C homogeneous assays, thereby resulting in lower estimations of LDL-C. Further studies must examine the reactivity to small dense LDL in LDL-C homogeneous assays.

When investigating the correlations of the 4 homogenous assays for LDL-C to the Friedewald equation, LDL-C (S), which is selective solubilization method for LDL-C, correlated most closely to the Friedewald equation and even with type III hyperlipoproteinemia samples, a favorable correlation was observed between LDL-C (S) and LDL-C (BQ), thus suggesting that LDL-C (S) closely reflects the clinical databases for CHD where LDL-C was estimated by the Friedewald equation.

Acknowledgments

We thank all patients and study participants. We also thank Dr. Masakazu Nakamura (Osaka Medical Center for Health Science and Promotion in Osaka, Japan, CDC/CRMLN Lipid Reference Laboratory) for the measurement of the β-quantification method.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cca.2009.09.010.

References

[1] Grundy SM. Role of low-density lipoproteins in atherogenesis and development of coronary heart disease. Clin Chem 1995;41:139–46.
[2] Gordon T, Kannel WB, Castelli WP, Dawber TR. Lipoproteins, cardiovascular disease and death. The Framingham study. Arch Interm Med 1981;141: 1128–30.
[3] 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.
[4] Hatch FT, Lees RS. Practical methods for plasma lipoprotein analysis. Adv Lipid Res 1968;6:1–68.
[5] Bachorik PS. Measurement of low-density-lipoprotein cholesterol. In: Rifai N, Warnick GR, Dominiczak MH, editors. Handbook of Lipoprotein Testing. 2nd ed. Washington, DC: AACC Press; 2000. p. 245–65.
[6] Nauck M, Wiebe D, Warnick GR. Measurement of high-density-lipoprotein cholesterol. In: Rifai N, Warnick GR, Dominiczak MH, editors. Handbook of Lipoprotein Testing. 2nd ed. Washington, DC: AACC Press; 2000. p. 221–44.
[7] Bachorik PS, Ross JW. National cholesterol education program recommendations for measurement of low-density lipoprotein cholesterol: executive summary. The national cholesterol education program working group on lipoprotein measurement. Clin Chem 1995;41:1414–20.
[8] Myers GL, Cooper GR, Greenberg N, Kimberly MM, Waymack PP, Hassemer DJ. Standardization of lipid and lipoprotein measurements. In: Rifai N, Warnick GR, Dominiczak MH, editors. Handbook of Lipoprotein Testing. 2nd ed. Washington, DC: AACC Press; 2000. p. 717–48.
[9] 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.
[10] Nauck M, Winkler K, März W, Wieland H. Quantitative determination of high-, low-, and very-low-density lipoproteins and lipoprotein (a) by agarose gel electrophoresis and enzymatic cholesterol staining. Clin Chem 1995;41:1761–7.
[11] Carroll RM, Rudel LL. Lipoprotein separation and low density lipoprotein molecular weight determination using high performance gel-filtration chromatography. J Lipid Res 1983;24:200–7.
[12] Kramer MA. Forcus on cholesterol research. New York: Nova Science Publishers, Inc.; 2005. 113–146.
[13] Hainline A, Jr, Karon J, Lippel K, eds. Manual of labolatory operations: lipidand lipoprotein analysis, 2ed ed. [HEW Pub. No. (NIH) 75–628 (rev.), U.S. Government Printing Office Publication No. 1982–361–132:678.] Bethesda, MD: National Heart, Lung, and Blood Institute, Lipid Research Clinics Program.
[14] Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz Jr ID. Measurement of low-density lipoprotein cholesterol in serum: a status report. Clin Chem 1992;38:150–60.
[15] Wilson P, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998;97:1837–47.
[16] Cobain MR, Pencina MJ, D'Agostino RB, Vasan RS. Lifetime risk for developing dyslipidemia: the Framingham offspring study. Am J Med 2007;120:623–30.
[17] Nauck M, Warnick GR, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. Clin Chem 2002;48:236–54.
[18] Hodis HN, Mack WJ, Dunn M, Liu C, Selzer RH, Krauss RM. Intermediate-density lipoproteins and progression of carotid arterial wall intima-media thickness. Circulation 1997;95:2022–6.
[19] Nauck M, Graziani MS, Bruton D, Cobbaert C, Cole TG, Lefevre F, et al. Analytical and clinical performance of a detergent-based homogeneous LDL-cholesterol assay: a multicenter evaluation. Clin Chem 2000;46:506–14.
[20] Fei H, Maeda S, Kirii H, Fujigaki S, Maekawa N, Fujii H, et al. Evaluation of two different homogeneous assays for LDL-cholesterol in lipoprotein-X-positive serum. Clin Chem 2000;46:1351–6.
[21] Ordóñez-Llanos J, Wägner AM, Bonet-Marqués R, Sánchez-Quesada JL, Blanco-Vaca F, González-Sastre F. Which cholesterol are we measuring with the Roche direct, homogeneous LDL-C plus assay? Clin Chem 2001;47:124–6.
[22] Iwasaki Y, Matsuyama H, Nakashima N. Improved specificity of a new homogeneous assay for LDL-cholesterol in serum with abnormal lipoproteins. Clin Chem 2006;52:886–8.
[23] Kameda K, Matsuzawa Y, Kubo M, Ishikawa K, Maejima I, Yamamura T, et al. Increased frequency of lipoprotein disorders similar to type III hyperlipoproteinemia in survivors of myocardial infarction in Japan. Atherosclerosis 1984;51: 241–9.
[24] Mishima Y, Ando M, Kuyama A, Ishioka T, Kibata M. A simple method for identifying particle size of low-density lipoprotein using PAG electrophoresis: comparison between LipoPhorTM and LipoPrint LDLTM systems. J Jpn Atheroscler Soc 1997;25:67–70.
[25] Hirano T, Ito Y, Yoshino G. Measurement of small dense low-density lipoprotein particles. J Atheroscler Thromb 2004;12:67–72.
[26] Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–10.
[27] Yanai H. Statcel: the useful add-in software forms on Excel. 2nd ed. Tokyo: OMS; 2004.
[28] Wikinski RL, Schreier LE, Rosental SB. New method for isolating and quantifying intermediate and β-very-low-density lipoprotein cholesterol. Clin Chem 1991;37: 1913–6.
[29] Mahley RW, Rall Jr SC. Type III hyperlipoproteinemia (Dysbetalipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: Scriver CR, Beaudet AL, Sly WS, editors. The metabolism and molecular bases of inherited disease. 8th ed. New York: McGraw-Hill; 2001. p. 2835–62.

[30] Todo Y, Kobayashi J, Higashikata T, Kawashiri M, Nohara A, Inazu A, et al. Detailed analysis of serum lipids and lipoproteins from Japanese type III hyperlipoproteinemia with apolipoprotein E2/2 phenotype. Clin Chim Acta 2004;348:35–40.
[31] Vergès B, Florentin E, Baillot-Rudoni S, Monier S, Petit JM, Rageot D, et al. Effect of 20 mg rosuvastatin on VLDL1-, VLDL2-, IDL- and LDL-ApoB kinetics in type 2 diabetes. Diabetologia 2008;51:1382–90.
[32] Havel RJ, Kane JP. Introduction: structure and meabolism of pasma lioproteins. In: Scriver CR, Beaudet AL, Sly WS, editors. The Metabolism and Molecular Bases of Inherited Disease. 8th ed. New Tork: McGraw-Hill; 2001. p. 2705–16.
[33] Sugiuchi H, Irie T, Uji Y, Ueno T, Chaen T, Uekama K, et al. Homogeneous assay for measuring low-density lipoprotein cholesterol in serum with triblock copolymer and α-cyclodextrin sulfate. Clin Chem 1998;44:522–31.
[34] Miller WG, Waymack PP, Anderson P, Ethridge SF, Jayne E. Performance of 4 homogeneous direct methods for LDL-cholesterol. Clin Chem 2002;48:489–98.
[35] Yanagi K, Yamashita S, Kihara S, Nakamura T, Nozaki S, Nagai Y, et al. Characteristics of coronary artery disease and lipoprotein abnormalities in patients with heterozygous familial hypercholesterolemia associated with diabetes mellitus or impaired glucose tolerance. Atherosclerosis 1997;132:43–51.
[36] Krauss RM, Lindgren FT, Williams PT, Kelsey SF, Brensike J, Vranizan K, et al. Intermediate-density lipoproteins and progression of coronary artery disease in hypercholesterolaemic men. Lancet 1987;2:62–6.
[37] Phillips NR, Waters D, Havel RJ. Plasma lipoproteins and progression of coronary artery disease evaluated by angiography and clinical events. Circulation 1993;88:2762–70.
[38] Galeano NF, Milne R, Marcel YL, Walsh MT, Levy E, Ngu'yen TD, et al. Apoprotein B structure and receptor recognition of triglyceride-rich low density lipoprotein (LDL) in modified in small LDL, but not in triglyceride rich LDL of normal size. J Biol Chem 1994;269:511–9.
[39] McNamara JR, Small DM, Li Z, Schaefer EJ. Differences in LDL subspecies involve alterations in lipid composition and conformational changes in apolipoprotein B. J Lipid Res 1996;37:1924–35.
[40] De Graaf J, Hak-Lammers HL, Hectors MP, Demacker PN, Hendriks JC, Stalenhoef AF. Enhanced susceptibility to in vivo oxidation of the dense low density lipoprotein subfraction in heasthy subjects. Arterioscler Thromb 1991;11:298–306.
[41] Dejager S, Bruckert E, Chapman MJ. Dense low density lipoprotein subspecies with diminished oxidative resistance predominate in combined hyperlipidemia. J Lipid Res 1993;34:295–308.

Archives of Gerontology and Geriatrics 49 Suppl. 2 (2009) S1–S2

Contents lists available at ScienceDirect

Archives of Gerontology and Geriatrics

journal homepage: www.elsevier.com/locate/archger

Geriatrics in the most aged country, Japan

Hidenori Arai*

Department of Human Health Sciences, Kyoto University Graduate School of Medicine, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

ARTICLE INFO

Keywords:
Aging society
Long-term care insurance
Comprehensive geriatric assessment
Health care system

ABSTRACT

The aging of society is a common problem in many developed countries. To tackle the problems related to an aging society, the role of geriatricians, as well as government support, is becoming more and more important. There is need to recruit young physicians with the skills required to care for elderly patients, and to establish an education system which encourages more young physicians to undertake training in geriatrics. Additionally, there is a need for improvement of our insurance systems to support such care. Our approach to the aging society will pave the way for other countries in Asia and the west.

1. Aging of society in Japan

The aging of society is a phenomenon affecting many developed countries in the west and Asia (Khaw, 1999; Imuta et al., 2001). Among them, Japan has the highest proportion of over-65s in the world: 22.1% in 2008. Further, 10.4% of the total population is over 75, making Japan the first country to have over 10% of the population in this very elderly range. In addition, we have also the longest average life span in the world: the 2008 life span being approximately 79 and 86, in men and women respectively. The aging rate will continue to rise in the future, yet fewer babies are born each year. Accordingly, Japan is inexorably aging. Our population has already peaked just short of 130 million, and we will face a population decline on a scale unprecedented in the developed world during this century. Although we have obtained this astonishing longevity, relatively inexpensively and within a short period of time, compared with other countries, we are facing a lot of problems associated with aging of our society, such as shortage of health-care professionals. Another problem is the increase of people with cognitive decline or dementia. The number of such people is estimated to be approximately 2 million and will definitely increase in the future (Meguro et al., 2007; Ikejima et al., 2009). Therefore, the support for dementia patients and their family becomes more and more important.

Our economic growth rate and technological achievements held the world in awe after World War II. However, in the future, Asian, as well as Western countries should watch Japan for another reason. The choices we make can affect the way in which the rest of the aging world tackles the same problem in years to come. Therefore, in this editorial I will describe what we have done for our society and what we should do as geriatricians and as members of the Japan Geriatrics Society, to meet the health-care needs of our aging population.

2. Establishment of long-term care insurance (LTCI)

One of the problems tackled was to develop a social insurance system for elderly people, especially for frail elderly, mainly for the purpose of community-based caregiving. Japan moved decisively toward "socialization of care" for the frail elderly by initiating public, mandatory long-term care insurance (LTCI) on April 1, 2000 (Arai, 2001). Those aged 65 and over who need nursing care in their daily lives and those who require long-term care due to illnesses between the ages of 40 and 64 (specified illnesses) are eligible to receive the care services of LTCI. Consumers can choose the services and providers that they want. However, the increase in the number of elderly people requiring LTCI, and the associated costs of these services, are imposing a burden on our society. The Ministry of Health, Labor and Welfare estimated that the number of elderly people requiring LTCI will increase from 1.5 million in 2000 to 5.2 million by 2025. This increase in LTCI demand due to an increase in the elderly population will jeopardize our society, as well as the LTCI service itself. It has been reported that most institutions and businesses related to LTCI face difficulties in their operation. Most such care facilities employ care workers bettering their running. As care workers in the LTCI service receive insufficient rewards for their efforts, there is a high turnover rate, with many such workers leaving their jobs. Although the relationships between the economy and labor situation, and increases in LTCI demand are not clear, stable management of LTCI, including the efficient running of related facilities, and high job satisfaction among care workers, is required for the sustainability of the LTCI system. Since 2006, LTCI regional comprehensive support centers have been introduced to deal with counseling related to elderly care and care management. Nurses, care workers, and physical and occupational therapists are running these centers. In spite of tough economic situations, I hope the Japanese government will decide to put more money into this system, although we should maintain our efforts to improve the cost-effectiveness of health care for the elderly, and decrease the number of frail elderly by preventing cardiovascular

* Correspondence: Tel./fax: +(81-75)751 3861.
E-mail address: harai@kuhp.kyoto-u.ac.jp (H. Arai).

0167-4943 /$ – see front matter