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Impact of low-density lipoprotein particle size on carotid intima-media thickness in patients with type 2 diabetes mellitus

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Abstract

Small low-density lipoprotein (LDL) particles and modifications to LDL such as glycation and oxidation have been linked to the pathogenesis of atherosclerosis in patients with diabetes. We investigated whether LDL particle size, or the levels of glycated LDL or malondialdehyde-modified LDL (MDA-LDL) are associated with carotid intima-media thickness (IMT) in patients with type 2 diabetes mellitus. One hundred seventy-two patients with type 2 diabetes mellitus were enrolled. Carotid IMT was measured by high-resolution ultrasound, and LDL particle size and serum glycated LDL and MDA-LDL levels were determined. The 3 variables were significantly correlated with one another. Univariate analyses defined statistically significant correlations of carotid IMT with LDL size, hemoglobin A_{1c}, glycated LDL, MDA-LDL, high-density lipoprotein (HDL) cholesterol, and age. The strongest association of IMT was with LDL size ($r = -0.406$, $P < .0001$), followed by that with HDL cholesterol ($r = -0.225$, $P = .004$). A stepwise multiple regression analysis revealed that LDL size and HDL cholesterol are independent predictors of carotid IMT. Neither glycated LDL nor MDA-LDL had a significant independent contribution to the severity of carotid IMT in the multivariate model. Low-density lipoprotein particle size, but not the glycated LDL or MDA-LDL level, was independently associated with carotid IMT in patients with type 2 diabetes mellitus regardless of antidiabetic and lipid-lowering medications. These results suggest that the measurement of LDL size may be more useful than quantification of modified LDLs for assessing atherosclerosis in patients with type 2 diabetes mellitus. Small LDL particles may be the most important predictor for the risk of cardiovascular disease in diabetic patients.

1. Introduction

Low-density lipoprotein (LDL) cholesterol has been widely recognized as a strong predictor of cardiovascular disease (CVD). Low-density lipoprotein is heterogeneous in terms of size, density, and chemical composition [1]. Small LDL particles are particularly prone to oxidation, providing a possible mechanism for their atherogenicity [2]. Therefore, LDL particle size has been increasingly recognized as a potential marker of increased risk for CVD [3-5]. On the other hand, oxidative modification of LDL [6-8] and glycated LDL [9] have been linked to the development of atherosclerosis and the increased incidence of CVD and myocardial infarction. Type 2 diabetes mellitus is one of the most important risk factors associated with CVD [10]. The diabetic state has been associated with alterations in the characteristics of LDL particles such as increased oxidation and glycation [11]. Atherosclerosis is a major complication of diabetes mellitus and increases the risk for subsequent development of CVD and cerebrovascular diseases. These complications are the primary cause of mortality in diabetes. However, few studies have investigated the relationship of LDL modification with atherosclerosis in patients with type 2 diabetes mellitus [12].

High-resolution B-mode ultrasound is a reliable, noninvasive method for detecting early structural changes in the arterial wall. Increased carotid intima-media thickness (IMT) is a structural marker of atherosclerosis that correlates with vascular risk factors, relates to the extent of coronary artery disease, and predicts the likelihood of cardiovascular events [13,14]. The present study was aimed at investigating

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the association of carotid IMT with LDL particle size, oxidative modification of LDL, and glycated LDL.

2. Research design and methods

2.1. Study population

The study population consisted of 172 Japanese adults; 121 were inpatients and the remaining 51 were outpatients. They included 124 patients with CVD, but patients with recent myocardial infarction or acute coronary syndrome were excluded from the study. All the participants had type 2 diabetes mellitus (111 men and 61 women), with a mean age of 66.8 ± 10.3 years (mean ± SD) (range, 31-96 years). Diabetes mellitus was diagnosed according to World Health Organization 1999 criteria. Informed consent was obtained from all subjects. The local medical ethics committee approved the study, which was carried out in accordance with the Declaration of Helsinki.

2.2. Laboratory measurements

Measurements of total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and LDL cholesterol were performed on plasma collected after the subjects fasted overnight, using enzymatic methods (7350E; Hitachi, Mito, Japan). Hemoglobin A_{1c} (HbA_{1c}) was measured by ion exchange high-performance liquid chromatography (Hemoglobin A_{1c} Variant; Bio-Rad Laboratories, Munich, Germany), with normal values ranging from 4.6% to 5.8%. Insulin levels were measured by the enzyme immunoassay method. Serum high-sensitivity C-reactive protein (hs-CRP) concentration was measured by an automatic immunonephelometer.

The homeostasis model assessment of insulin resistance (HOMA-IR) calculated from fasting glucose and insulin concentrations was used as insulin resistance [15].

2.3. Low-density lipoprotein particle size

EDTA plasma samples were stored frozen at −70°C until analyzed. The LDL particle size was determined by electrophoresis using nondenaturing 4% to 14% polyacrylamide gradient gels with modified methods [16] as originally described by Krauss and Burke [17]. In brief, 7.5 μL of plasma samples were applied on gels for a final concentration of 20% sucrose and 0.25% bromphenol blue. After electrophoresis, the gels were scanned (CS9300; Shimadzu, Kyoto, Japan) and migration distances (from the top of the gel to the most prominent band) were measured. The apparent diameters of major LDL particles were measured by comparing results with a calibration curve constructed with ferritin, thyroglobulin, and latex beads. The estimated diameter for the major peak in each scan was identified as the LDL particle size.

2.4. Malondialdehyde-modified LDL

The enzyme-linked immunosorbent assay used for measurement of malondialdehyde-modified LDL (MDA-LDL) was based on the method reported by Kotani et al [18]. In brief, microtiter plates were coated with a monoclonal antibody against MDA-LDL. Duplicate samples, each 100 μL, were added to the wells of the plates and incubated for 1 hour at room temperature. After washing, a β-galactosidase–conjugated monoclonal antibody against apolipoprotein B (apo B) was added to each well, and the mixture was incubated for 30 minutes at room temperature. After washing, 100 μL of the substrate solution, *o*-nitrophenyl-β-galactopyranoside (10 mmol/L), was dispensed into each well and allowed to react for 2 hours at room temperature. The reaction was terminated by stop solution, and absorbance was determined spectrophotometrically at 415 nm. The primary standard used was artificially prepared MDA-LDL in which 15% of the amino groups were modified. From a curve constructed using this standard, the amount of MDA-LDL in the samples was determined. One unit per liter was defined as the absorbance obtained with the standard at a concentration of 1 mg/L.

2.5. Glycated LDL

Quantification of glycated LDL was performed with a commercial kit (Glycacor, Exocell, Philadelphia, PA). The competitive enzyme-linked immunosorbent assay used a mouse monoclonal antibody that recognizes a specific epitope on glycated apo B in the LDL complex [19]. Glycated LDL in the solid phase was detected with horseradish peroxidase–conjugated goat antimouse antibody based on color reaction. The absorbance was determined in a microplate reader at 450 nm. The concentration in a patient sample was determined from a simultaneously run standard curve with glycated apo B diluted serially from a lyophilized preparation of human glycated LDL of known concentration.

2.6. Carotid intima-media thickness assessment

High-resolution B-mode carotid ultrasonography was performed by using a SONOS 5500 (Philips Medical Systems, Best, the Netherlands) with a linear-array 3- to 11-MHz transducer. Carotid ultrasound studies were performed by a single technician. The subjects lay in the supine position in a dark, quiet room. The right and left common carotid arteries (CCAs) were examined with the subject's head tilted slightly upward in the midline position. The transducer was manipulated so that the near and far walls of the CCA were parallel to the transducer footprint and the lumen diameter was maximized in the longitudinal plane. A single observer blinded to subjects' vascular risk measured the combined thickness of intima and media of the far wall of both CCAs. For each patient, the largest IMT among the values for the right and left CCA, carotid bulb, and internal carotid artery was chosen as the outcome variable [20].

2.7. Statistical analysis

Data are presented as means ± SD. Pearson correlation coefficient (r) was used for correlations between

Table 1
Clinical characteristics of study subjects

		Range
No. of patients	172	
Male/Female	111/61	
Age (y)	66.8 ± 10.3	31-96
Lipid-lowering medications (yes/no)	85/87	
Diabetes medications (yes/no)	70/102	
BMI (kg/m^2)	23.0 ± 3.9	14.3-44.1
Systolic blood pressure (mm Hg)	135 ± 22	80-201
Diastolic blood pressure (mm Hg)	77 ± 13	46-106
Total cholesterol (mmol/L)	4.86 ± 1.01	3.03-8.64
HDL cholesterol (mmol/L)	1.25 ± 0.37	0.60-2.40
LDL cholesterol (mmol/L)	2.83 ± 0.93	1.44-6.91
Triglycerides (mmol/L)	1.59 ± 1.16	0.50-10.03
HbA_{1c} (%)	6.3 ± 1.2	4.1-12.8
Fasting glucose (mmol/L)	7.2 ± 2.6	4.0-18.6
Insulin (pmol/L)	59 ± 77	6.6-624
HOMA-IR	3.3 ± 4.7	0.3-32.4
LDL particle size (nm)	25.1 ± 1.9	20.7-29.0
Glycated LDL (mg/dL)	2.43 ± 0.62	1.15-4.96
MDA-LDL (IU/L)	132.8 ± 72.1	28.5-464.6
hs-CRP (mg/dL)	0.284 ± 0.588	0.000-4.500
IMT(mm)	1.1 ± 0.4	0.5-2.6

Data are presented as mean ± SD unless otherwise indicated.

continuous variables and the Spearman correlation coefficient (ρ) was applied for correlations with sex. Variables associated with IMT in the univariate analyses ($P < .10$) were then included in a multivariate analysis. Forward stepwise multiple regression analysis was performed including all variables given in Table 2 to examine significant contributions of variables to the prediction of the maximum IMT. Sex was entered as female = 1, male = 0. All statistical analyses were performed using StatView 5.0 (SAS Institute, Cary, NC). A level of $P < .05$ was accepted as statistically significant.

3. Results

3.1. Characteristics of the subjects

Baseline characteristics of the subjects in this study are shown in Table 1. The patients had received antidiabetic treatment (insulin therapy, 26; oral antidiabetic agents, 49; total, 70) and lipid-lowering agents (atorvastatin, 30; fluvastatin, 27; pravastatin, 10; simbastatin, 13; bezafibrate, 5; total, 85), which potentially could influence circulating lipid profiles. The mean levels of fasting glucose and HbA_{1c} were 7.2 ± 2.6 mmol/L (range, 4.0-18.6 mmol/L) and 6.3% ± 1.2% (range, 4.1%-12.8%), respectively, because some patients had good glycemic control by antidiabetic medication and some were poorly controlled. We found that there was a significant difference in LDL particle size according to lipid-lowering medications (24.8 ± 1.9 and 25.5 ± 1.9 nm in the presence and absence of lipid-lowering medications, respectively; $P = .013$) and in glycated LDL according to diabetes medications (2.55 ± 0.74 and 2.34 ± 0.49 mg/dL in the presence and absence of diabetes medications, respectively; $P = .017$).

3.2. Association of IMT with lipoproteins and other risk factors

Table 2 shows the correlation of IMT with lipoproteins and other risk factors. Intima-media thickness was significantly correlated with age ($r = 0.157$, $P = .044$), HDL cholesterol ($r = -0.225$, $P = .0037$), HbA_{1c} ($r = 0.182$, $P = .019$), LDL particle size ($r = -0.406$, $P < .0001$), glycated LDL ($r = 0.166$, $P = .036$), and MDA-LDL ($r = 0.162$, $P = .040$). The strongest association of IMT was observed with LDL particle size, followed by the association with HDL cholesterol (Fig. 1). However, there was no significant association with total cholesterol, LDL cholesterol, triglyceride, or fasting glucose levels.

Although LDL particle size was the most strongly associated with IMT, neither the glycated LDL nor the MDA-LDL level had a significant independent contribution to the severity of carotid IMT in the multivariate model using variables with $P < .10$ at the univariate analysis. A stepwise multiple regression analysis revealed that LDL particle size and HDL-C were independent predictors of carotid IMT (total $R^2 = 0.165$).

3.3. Association of LDL particle characteristics with lipoproteins and other risk factors

In addition to the association with IMT, the correlation analyses revealed that LDL particle size was significantly correlated with glycated LDL and MDA-LDL levels (Table 3). The glycated LDL levels reflected the concentration of glucose comparatively well, on the basis of a close relationship with fasting glucose and HbA_{1c}. In addition to the association with LDL particle size, glycated LDL was

Table 2
Correlation of carotid IMT with lipoprotein levels and other risk factors in type 2 diabetes mellitus

	Univariate analysis		Multivariate analysis[a]	
	r or ρ	P	β	P
Age	0.157	.044	0.145	.047
Sex	−0.152	.052	−0.147	.054
BMI	−0.058	.46		
Systolic blood pressure	0.072	.36		
Diastolic blood pressure	0.077	.33		
Total cholesterol	0.018	.82		
HDL cholesterol	−0.225	.004	−0.145	.048
LDL cholesterol	0.039	.62		
Triglycerides	0.012	.88		
HbA_{1c}	0.182	.019	0.125	.098
Fasting glucose	0.035	.65		
Insulin	−0.041	.60		
HOMA-IR	−0.014	.85		
LDL particle size	−0.406	<.0001	−0.315	<.0001
Glycated LDL	0.166	.036	0.103	.19
MDA-LDL	0.162	.040	0.111	.13
hs-CRP	0.018	.17		

r indicates linear (Pearson) correlation coefficient; ρ, nonparametric (Spearman) correlation coefficient; β, partial regression coefficient.

[a] Only variables with $P < .10$ at the univariate analysis were entered into the multivariate analysis.

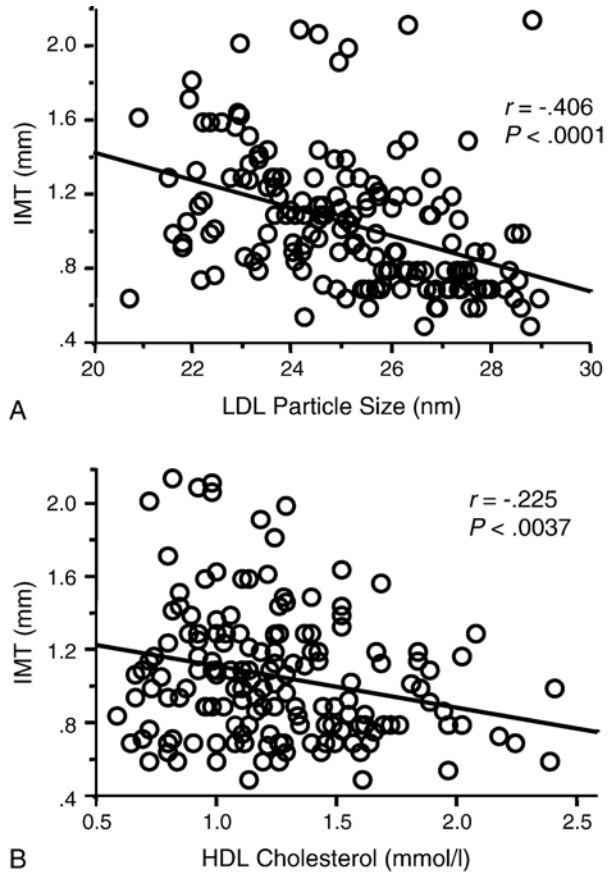

Fig. 1. Scatterplot and correlation of carotid IMT with LDL particle size (A) and HDL cholesterol (B) in type 2 diabetes mellitus (N = 172).

also associated with MDA-LDL, age, and total cholesterol. In addition to the significant correlation shown above, the MDA-LDL level was also significantly correlated with total cholesterol and sex (Table 3).

4. Discussion

Patients with type 2 diabetes mellitus are at high risk for cardiovascular morbidity and mortality. There is considerable interest in defining factors responsible for the accelerated development of atherosclerosis in individuals with diabetes. Evaluation of carotid IMT using high-resolution ultrasound may precede CVD [13,14]. Carotid IMT is a structural marker of early atherosclerosis that correlates with vascular risk factors and relates to the severity and extent of CVD [21,22]. So far, LDL cholesterol has been shown to be correlated with IMT [23], but there was no association in this study probably because of lipid-lowering medications.

The present study demonstrated that severity of carotid IMT is significantly associated with small LDL particle size and is also related with circulating levels of glycated LDL and MDA-LDL. Increased LDL cholesterol levels are now well established as a major risk factor for CVD. Low-density lipoprotein particles vary in size and hydrated density [1]. Small LDL particles have been shown to be more atherogenic than larger LDL particles in cross-sectional [3,4] and prospective [24] studies, and there is growing evidence to support a correlation between a predominance of small LDL and the risk of coronary artery disease.

Plasma triglyceride levels have been identified as the single best metabolic correlate of LDL particle size in several populations [3,24]. In the present study, however, the major reason for a lack of association with triglyceride levels is that the recruited patients received antidiabetic and antihyperlipidemic medications, because we could not enroll a sufficient number of subjects with overt diabetes who were not taking medication. Insulin resistance, which is closely associated with plasma triglycerides [25], is reportedly related to reduced LDL particle size [26], but there was no significant association with HOMA-IR in this study.

Previous studies have shown that LDL size is significantly lower in diabetic than in nondiabetic subjects. However, it has been reported that the effect of diabetes on LDL size is not significant after adjustment for diabetic dyslipidemia because diabetic dyslipidemia greatly influences the LDL size distribution [27]. On the other hand, in the case of approximately 10-kg weight loss, reduced body fat and concomitant decreases in plasma triglyceride and insulin levels, LDL particle size remained unchanged to some extent, particularly in patients with normal-size LDL particles [28]. Despite the lack of association with triglyceride levels, LDL particle size still maintained a significant correlation with glycated LDL and MDA-LDL levels, indicating that these 3 circulating LDL profiles may contribute to the increased risk of atherosclerosis in patients with type 2 diabetes mellitus. Notably, multiple regression analysis demonstrated that of the 3 variables, only LDL particle size is an independent predictor of severity of carotid IMT.

We have previously reported the good correlation of LDL size with glycated LDL levels in subjects without medication [29]. The glycated LDL level was more strongly associated with plasma glucose levels because its correlation with fasting glucose levels and HbA_{1c} was much higher than

Table 3
Correlations among LDL particle size, glycated LDL level, and MDA-LDL level, and of each value with other risk factors in type 2 diabetes mellitus

	r or ρ	P
LDL particle size		
Glycated LDL	−0.163	.039
MDA-LDL	−0.161	.041
Glycated LDL		
Fasting glucose	0.330	<.0001
HbA_{1c}	0.327	<.0001
Age	−0.212	.0068
MDL-LDL	0.179	.023
Total cholesterol	0.167	.034
MDA-LDL		
Total cholesterol	0.424	<.0001
Sex	0.209	.0075

r indicates linear (Pearson) correlation coefficient; ρ, nonparametric (Spearman) correlation coefficient.

that with LDL particle size. In this study, there was no association of LDL particle size with fasting glucose levels and HbA_{1c}.

Oxidation of LDL contributes to the development of atherosclerosis. Malondialdehyde-modified LDL is recognized as a surrogate marker of oxidized LDL, and it has been suggested that the circulating MDA-LDL level could be a useful indicator for the identification of patients with CVD. In the present study, however, the best correlation with severity of IMT in patients with type 2 diabetes mellitus was LDL particle size, not MDA-LDL. These results may be attributed to antidiabetic and lipid-lowering medications. In addition to the antidiabetic treatment of 70 patients, half of the participants in this study had been given statins including stronger statins such as atorvastatin. Statins have a considerable effect on lowering small LDL particle levels [30] and have also been proven to promote systemic antioxidant effects in vivo through suppression of different oxidation pathways, including the generation of myeloperoxidase-derived and nitric oxide–derived oxidants [31]. Based on our results, therefore, it is possible that statins have more beneficial effects on MDA-LDL than on LDL particles. Statins may inhibit oxidation, resulting in lowering of MDA-LDL production (121 ± 69 and 140 ± 73 IU/L in the presence and absence of lipid-lowering medications, respectively; $P = .09$). Furthermore, because LDL size is considerably influenced by genetic factors such as cholesteryl ester transfer protein [32] and β_3-adrenergic receptor genes [33], LDL size seems to be more resistant to modification by medication and environmental factors. Evaluation of small LDL particles is very important in diabetes as well as in nondiabetes.

Epidemiologic studies demonstrated that Japanese subjects with a lower insulin response to glucose show a higher risk for later development of type 2 diabetes mellitus. Namely, the insulin secretory defect is a primary metabolic defect in Japanese [34]. Although morbid obesity by Western Europe standards is rare in Japanese [35], studies of Japanese Americans have shown that visceral adiposity can vary up to 10-fold even in lean subjects and is a strong predictor of insulin resistance and onset of type 2 diabetes mellitus. Thus, Japanese patients with type 2 diabetes mellitus might have specific population differences influencing fat patterning and LDL particle characteristics. Therefore, our results may not be consistent with those obtained from white populations. In addition, that the prevalence of CVD is higher in the population with diabetes would be a limitation in this study.

In conclusion, LDL particle size, not the level of glycated LDL or MDA-LDL, was independently associated with carotid IMT in patients with type 2 diabetes mellitus regardless of antidiabetic and lipid-lowering medications, although LDL particle size, glycated LDL, and MDA-LDL were significantly associated with one another. These results suggest that the measurement of LDL particle size may be superior to that of modified LDL quantification for the assessment of the risk of CVD in patients with type 2 diabetes mellitus. Small LDL particles may be the most important predictor for the risk of CVD in diabetic patients.

References

[1] Shen MM, Krauss RM, Lindgren FT, Forte TM. Heterogeneity of serum low density lipoproteins in normal human subjects. J Lipid Res 1981;22:236-44.
[2] Chancharme L, Therond P, Nigon F, Lepage S, Couturier M, Chapman MJ. Cholesteryl ester hydroperoxide lability is a key feature of the oxidative susceptibility of small, dense LDL. Arterioscler Thromb Vasc Biol 1999;19:810-20.
[3] Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988;260:1917-21.
[4] Campos H, Genest Jr JJ, Blijlevens E, et al. Low density lipoprotein particle size and coronary artery disease. Arterioscler Thromb 1992; 12:187-95.
[5] Erbey JR, Robbins D, Forrest KY, Orchard TJ. Low density lipoprotein size and coronary disease in a childhood-onset type 1 diabetes population. Metabolism 1999;48:531-4.
[6] Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989;320:915-24.
[7] Berliner JA, Heinecke JW. The role of oxidized lipoproteins in atherogenesis. Free Radic Biol Med 1996;20:707-27.
[8] Liu ML, Ylitalo K, Salonen R, Salonen JT, Taskinen MR. Circulating oxidized low-density lipoprotein and its association with carotid intima-media thickness in asymptomatic members of familial combined hyperlipidemia families. Arterioscler Thromb Vasc Biol 2004;24:1492-7.
[9] Graier WF, Kostner GM. Glycated low-density lipoprotein and atherogenesis: the missing link between diabetes mellitus and hypercholesterolaemia? Eur J Clin Invest 1997;27:457-9.
[10] Kannel WB. Lipids, diabetes, and coronary heart disease: insights from the Framingham Study. Am Heart J 1985;110:1100-7.
[11] Moro E, Alessandrini P, Zambon C, et al. Is glycation of low density lipoproteins in patients with type 2 diabetes mellitus a LDL pre-oxidative condition? Diabet Med 1999;16:663-9.
[12] Berneis K, Jeanneret C, Muser J, Felix B, Miserez AR. Low-density lipoprotein size and subclasses are markers of clinically apparent and non-apparent atherosclerosis in type 2 diabetes. Metabolism 2005; 54:227-34.
[13] Salonen JT, Salonen R. Ultrasound B-mode imaging in observational studies of atherosclerotic progression. Circulation 1993; 87(3 Suppl):II56-II65.
[14] Chambless LE, Heiss G, Folsom AR, et al. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. Am J Epidemiol 1997;146:483-94.
[15] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment; insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
[16] Okumura K, Matsui H, Kawakami K, et al. Low density lipoprotein particle size is associated with glycosylated hemoglobin levels regardless of plasma lipid levels. Intern Med 1998;37:273-9.
[17] Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res 1982;23:97-104.
[18] Kotani K, Maekawa M, Kanno T, Kondo A, Toda N, Manabe M. Distribution of immunoreactive malondialdehyde-modified low-den-

sity lipoprotein in human serum. Biochim Biophys Acta 1994;1215: 121-5.
[19] Shea EA, Cohen MP. Immunologic detection and measurement of glycated apolipoprotein B with site specific monoclonal antibodies. J Immunol Methods 1993;162:85-95.
[20] Baldassarre D, Amato M, Pustina L, et al. Increased carotid artery intima-media thickness in subjects with primary hypoalphalipoproteinemia. Arterioscler Thromb Vasc Biol 2002;22:317-22.
[21] Kodama M, Yamasaki Y, Sakamoto K, et al. Antiplatelet drugs attenuate progression of carotid intima-media thickness in subjects with type 2 diabetes. Thromb Res 2000;97:239-45.
[22] Hosomi N, Mizushige K, Ohyama H, et al. Angiotensin-converting enzyme inhibition with enalapril slows progressive intima-media thickening of the common carotid artery in patients with non–insulin-dependent diabetes mellitus. Stroke 2001;32:1539-45.
[23] Goya K, Kitamura T, Inaba M, et al. Risk factors for asymptomatic atherosclerosis in Japanese type 2 diabetic patients without diabetic microvascular complications. Metabolism 2003;52:1302-6.
[24] Lamarche B, Tchernof A, Moorjani S, et al. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men: prospective results from the Quebec cardiovascular study. Circulation 1997;95:69-75.
[25] Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. J Clin Invest 1993;92:141-6.
[26] Mykkanen L, Haffner SM, Rainwater DL, Karhapaa P, Miettinenn H, Laakso M. Relationship of LDL size to insulin sensitivity in normoglycemic men. Arterioscler Thromb Vasc Biol 1997;17: 1447-53.
[27] Singh AT, Rainwater DL, Haffner SM, et al. Effect of diabetes on lipoprotein size. Arterioscler Thromb Vasc Biol 1995;15: 1805-11.
[28] Katzel LI, Coon PJ, Rogus E, Krauss RM, Goldberg AP. Persistence of low HDL-C levels after weight reduction in older men with small LDL particles. Arterioscler Thromb Vasc Biol 1995;15:299-305.
[29] Matsui H, Okumura K, Toki Y, Hayakawa T. Low-density lipoprotein particle size as an independent predictor of glycated low-density lipoprotein level. Diabetes Care 1999;7:1220-1.
[30] Brousseau ME, Schaefer EJ, Wolfe ML, et al. Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. N Engl J Med 2004;350:1505-15.
[31] Shishehbor MH, Brennan ML, Aviles RJ, et al. Statins promote potent systemic antioxidant effects through specific inflammatory pathways. Circulation 2003;108:426-31.
[32] Okumura K, Matsui H, Kamiya H, Saburi Y, Hayashi K, Hayakawa T. Differential effect of two common polymorphisms in the cholesteryl ester transfer protein gene on low-density lipoprotein particle size. Atherosclerosis 2002;161:425-31.
[33] Okumura K, Matsui H, Ogawa Y, et al. The polymorphism of the beta3-adrenergic receptor gene is associated with reduced low-density lipoprotein particle size. Metabolism 2003;52:356-61.
[34] Kadowaki T, Miyake Y, Hagura R, et al. Risk factors for worsening to diabetes in subjects with impaired glucose tolerance. Diabetologia 1984;26:44-9.
[35] Sasahara M, Nishi M, Kawashima H, et al. Uncoupling protein 2 promoter polymorphism-866G/A affects its expression in β-cells and modulates clinical profiles of Japanese type 2 diabetic patients. Diabetes 2004;53:482-5.