Malondialdehyde-modified LDL-related variables are associated with diabetic kidney disease in type 2 diabetes

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Title

Malondialdehyde-modified LDL-related variables are associated with diabetic kidney disease in type 2 diabetes

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Abstract

Background and Aims: Oxidized low-density lipoprotein (oxLDL) causes the development of atherosclerosis and kidney injury. Although circulating oxLDL levels were reportedly increased in type 2 diabetic patients with macroalbuminuria, it remains unclear whether albuminuria or the reduced glomerular filtration rate (GFR) is independently associated with the circulating oxLDL level. This study aimed to elucidate the association between the stage of diabetic nephropathy and serum malondialdehyde-modified LDL (MDA-LDL) and the ratio of MDA-LDL to LDL-cholesterol (MDA-LDL/LDL).

Methods and Results: This retroactive cross-sectional study used data from 402 patients with type 2 diabetes. Patients undergoing hemodialysis were excluded. Serum MDA-LDL levels were significantly increased with increases in severity of albuminuria (103 ± 44 U/L, 109 ± 54 U/L, and 135 ± 72 U/L for normoalbuminuria, microalbuminuria, and macroalbuminuria, respectively; P for trend = 0.020) but not according to the estimated GFR (eGFR). An increased MDA-LDL/LDL ratio was significantly associated with both increased albuminuria (35 ± 13, 37 ± 14, and 40 ± 15 for normoalbuminuria, microalbuminuria, and

macroalbuminuria, respectively; P for trend = 0.003) and reduced eGFR (34 ± 13, 36 ± 13, 38 ± 12, and 51 ± 28 for grade 1, 2, 3 and 4, respectively; P for trend = 0.002). Multiple linear regression analysis showed that neither the albumin excretion rate nor eGFR but ln-transformed triglycerides and LDL-C levels were independent determinants of both serum MDA-LDL levels and MDA-LDL/LDL ratios.

Conclusion: Serum MDA-LDL levels and MDA-LDL/LDL ratios were increased in those with dyslipidemia associated with diabetic kidney disease.

Keywords: malondialdehyde-modified low-density lipoprotein; type 2 diabetes; albuminuria; estimated glomerular filtration rate; dyslipidemia

Abbreviations

AER, albumin excretion rate: BMI, body mass index; CKD, chronic kidney disease; CVD, cardiovascular disease; DPP-4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; EPA, eicosapentaenoic

acid; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; ln AER, ln-transformed AER; ln HDL-C, ln-transformed HDL; ln TG, ln-transformed TG; MDA-LDL, malondialdehyde-modified low-density lipoprotein; MDA-LDL/LDL, MDA-LDL-to-LDL cholesterol ratio, oxLDL, oxidized low-density lipoprotein; TG, triglycerides

1. Background

Diabetes mellitus is a high-risk state for atherosclerosis, leading to increased morbidity and mortality due to cardiovascular disease (CVD). It is also widely accepted that chronic kidney disease (CKD) presents a substantial risk for CVD. The presence of both diabetes and CKD is associated with a higher incidence of myocardial infarction and all-cause mortality compared with diabetes or CKD alone [1].

Oxidized low-density lipoprotein (oxLDL) has a pivotal role in the initiation and progression of atherosclerosis. oxLDL elicits foam cell formation, leading to increased release of inflammatory cytokines and chemokines from foam cells. It also stimulates medial smooth muscle cell migration into the intima, which was shown to be an important process for intimal thickening in atherosclerotic lesions [2]. Serum malondialdehyde-modified LDL (MDA-LDL), which is a type of oxidized LDL, was reported as a prognostic marker for future cardiac events in patients with stable angina and coronary stent implantation [3] or in diabetic patients with CAD [4]. The MDA-LDL-to-LDL cholesterol ratio (MDA-LDL/LDL) could predict the presence and development of coronary artery disease [5, 6]. The MDA-LDL/LDL was independently

associated with the coronary artery calcification score in patients with hemodialysis [7].

Recent studies have shown that oxLDL can contribute to the development of diabetic nephropathy through inducing injuries to podocytes [8], tubulointerstitial cells [9] and endothelial cells [10]. Production of collagen IV in mesangial cells was also reported as a contributor to the progression of diabetic nephropathy, which is stimulated by oxLDL-containing immune complexes [9]. Immunoglobulin G antibodies reacting with MDA-LDL lysine epitopes in circulating immune complexes were noted as a predictor of the development of macroalbuminuria in patients with type 2 diabetes [11]. Circulating oxLDL was reportedly increased in albuminuria in patients with diabetic nephropathy as well as in patients with end-stage renal diseases [12-14]. Although albuminuria was negatively correlated with the estimated glomerular filtration rate (eGFR) [15], there have been no studies on the association between oxLDL and eGFR in diabetes. Moreover, it is not clear whether albuminuria or eGFR is independently associated with circulating oxLDL levels.

To elucidate the association between the stage of diabetic nephropathy and serum MDA-LDL or the MDA-LDL/LDL, we conducted a cross-sectional study of patients with type 2 diabetes.

2. Methods

2.1. Study participants

We conducted a retrospective cross-sectional study using data on patients with type 2 diabetes who were admitted to the University of Tsukuba Hospital from January 2012 to December 2015. All patients had undergone a structured interview, physical examination, and laboratory analysis. We excluded data on patients undergoing hemodialysis, with the complications of diabetic ketoacidosis, hyperglycemia hyperosmolar syndrome, viral hepatitis, liver cirrhosis, malignancy, endocrine disorders affecting serum lipid levels, infectious diseases, inflammatory diseases, pregnancy, renal diseases other than diabetic kidney disease, and the use of systemic glucocorticoids, and who were under 20 years of age. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or the current use of antihypertensive agents. This retrospective study was approved by the Ethics Committee of the University of Tsukuba Hospital and conducted according to the Declaration of Helsinki.

2.2. Laboratory Analysis

Blood samples were collected in the morning after an overnight fast within 3 days after admission. Plasma glucose and serum total cholesterol, high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), and creatinine levels were determined using an automated analyzer (7700 clinical analyzer; Hitachi High-Technologies Corporation, Tokyo, Japan). HbA1c was measured by high-performance liquid chromatography (Tosoh Corporation, Tokyo, Japan). Serum LDL-C levels were measured by a homogeneous assay (Sekisui Medical, Tokyo, Japan). Serum concentrations of MDA-LDL were quantitated by an enzyme-linked immunosorbent assay (Sekisui Medical, Tokyo, Japan). Albumin excretion rate (AER) was measured using 24-hour urine specimens. eGFR was calculated using an equation modified for the Japanese: eGFR = $194 \times sCre^{-1.0949} \times Age^{-0.287} \times 0.739$ (if female) [16]. AER and eGFR were categorized according to the 2012 Kidney Disease: Improving Global Outcomes clinical practice guidelines [17].

2.3. Statistical Analysis

Continuous variables were expressed as mean ± SD or median and interquartile range based on distribution. Values of TG, HDL-C, and AER were log transformed due to their non-normal distribution. MDA-LDL-

related variables (MDA-LDL and MDA-LDL/LDL) and lipid parameters were stratified by nephropathy categories (AER <30, ≥30<300, or ≥300 mg/day; eGFR ≥90, ≥60<90, ≥30<60, or <30 ml/min/1.73m^2) and were compared by the Kruskal–Wallis test because homogeneities of variance were not assumed for all variables. A Jonckheere–Terpastra test was used to analyze trends through categories. A Pearson's correlation coefficient or a Spearman's correlation coefficient was used to examine bivariate associations between MDA-LDL-related variables and clinical parameters, depending on distributions of the values of the parameters. A multiple linear regression analysis was performed to study independent determinants of MDA-LDL-related variables. A logistic regression analysis was conducted to examine whether MDA-LDL-related variables were independent predictors of the presence of CVD. Statistical analyses were performed using the Statistical Package for Social Science Statistics (version 23.0; Chicago, IL, USA). Statistical significance was considered at a P value of <0.05.

3. Results

3.1. Characteristics of Participants

Initially, data on 1,014 patients with type 1 and type 2 diabetes were examined; however, patients were excluded for the following reasons: undergoing hemodialysis (n = 6), diabetic ketoacidosis or hyperglycemia hyperosmolar syndrome (n = 40), acute illness (n = 88), perioperative period (n = 3), viral hepatitis and/or liver cirrhosis (n = 40), malignancy (n = 135), endocrine disorders affecting serum lipid levels (n = 121), inflammatory diseases (n = 24), pregnancy (n = 33), renal diseases other than diabetic kidney disease (n = 14), use of systemic glucocorticoids (n = 33), MDA-LDL and/or other lipid parameters not measured (n = 15), and age under 20 years (n = 6). After these exclusions, 456 patients were eligible for analysis. Of these, we analyzed data on 402 patients who had type 2 diabetes.

Table 1 shows the characteristics of the study participants. Their mean age was 57 ± 13 years, median duration of diabetes was 9.0 (3.0 - 16.0) years, mean body mass index (BMI) was 27.0 ± 5.4 kg/m^2, mean fasting plasma glucose (FPG) was 9.4 ± 2.7 mmol/L, and mean HbA1c level was 9.9 ± 1.9%. In terms of

renal function, the mean eGFR was 86.6 ± 29.5 mL/min/1.73m^2 and median AER was 13.8 (6.2 - 47.7) mg/day. The mean MDA-LDL value and MDA-LDL/LDL were 107 ± 50 U/L and 36 ± 14 [U/L]/[mmol/L], respectively.

Almost half of the patients were treated with sulfonylureas, metformin, and/or dipeptidyl peptidase-4 (DPP-4) inhibitors (41%, 41%, and 47%, respectively). Thirty-eight percent of patients were prescribed renin-angiotensin system inhibitors. Statins were used by 38% of patients and other lipid lowering drugs were prescribed for smaller groups (fibrate, ezetimib, and eicosapentaenoic acid [EPA] for 3%, 2%, and 3% of participants, respectively).

3.2. Associations between MDA-LDL-related variables and renal function

Table 2 shows the associations between the AER and lipid parameters. Serum MDA-LDL levels trended towards a positive association according to worsening of the category of albuminuria ($p = 0.006$, p for trend = 0.020), and the MDA-LDL/LDL showed a significant positive association as the category of albuminuria worsened ($p = 0.008$, p for trend = 0.003). As shown in Table 3, MDA-LDL levels did not differ significantly

among the categories of eGFR (p = 0.321, p for trend = 0.527). On the other hand, the MDA-LDL/LDL had a significant positive association with deterioration of the eGFR (p = 0.013, p for trend = 0.002).

3.3. Associations between MDA-LDL-related variables and clinical parameters

As shown in Table 4, MDA-LDL levels were positively correlated with diastolic blood pressure, FPG, HbA1c, ln-transformed AER (ln AER), ln-transformed TG (ln TG), and LDL (p = <0.001, 0.006, <0.001, <0.001, <0.001, and <0.001, respectively), whereas they were inversely correlated with age, taking statins, and ln-transformed HDL (ln HDL-C) (p = <0.001, <0.001, and 0.001, respectively). The levels of MDA-LDL/LDL had a positive correlation with hypertension, CVD, ln AER, statins, and ln TG (p = 0.003, <0.001, 0.002, <0.001, and <0.001, respectively), whereas there was an inverse correlation with eGFR, ln HDL-C, and LDL-C (p = 0.005, <0.001, and <0.001, respectively).

3.4. Multiple linear regression analyses of MDA-LDL-related variables

To determine the clinical parameters that were independently associated with MDA-LDL-related variables, a multiple linear regression analysis was performed that included the following variables: age, sex, CVD,

current smoking, BMI, hypertension, FPG, HbA1c, eGFR, ln AER, ln TG, ln HDL-C, LDL-C, taking statins and/or ezetimib, taking fibrates and/or EPA, and taking renin-angiotensin system inhibitors. Results showed that not AER nor eGFR but LDL-C and ln TG were independent determinants of both MDA-LDL and MDA-LDL/LDL (adjusted R^2 = 0.451 and 0.332, respectively) (Table 5).

3.5. Logistic regression analyses of MDA-LDL-related variables

Logistic regression analyses showed that MDA-LDL/LDL was an independent predictor of CVD but that MDA-LDL was not after adjustments for age, sex, duration of diabetes, hypertension, smoking, dyslipidemia, eGFR and AER (model 1) or age, sex, duration of diabetes, hypertension, smoking, dyslipidemia and diabetic kidney disease (model 2) (Supplemental Table 1).

4. Discussion

In this study, we examined the association between the stage of diabetic nephropathy and serum MDA-LDL-related variables. There were three major findings in the current study. First, serum MDA-LDL levels were significantly increased with increases in the severity of albuminuria but not with those of eGFR. Second, both increased albuminuria and reduced eGFR were significantly associated with an increased MDA-LDL/LDL. Third, multiple linear regression analyses showed that not albuminuria nor eGFR but ln TG and LDL-C levels were independent determinants of both serum MDA-LDL levels and the MDA-LDL/LDL.

Whether circulating oxLDL levels are increased in patients with type 2 diabetes with severe albuminuria is controversial. It was shown that serum oxLDL levels were increased in type 2 diabetic patients with macroalbuminuria [13, 18] which is consistent with this study, but one study could not find such an association [19]. Since AER levels are negatively correlated with eGFR and Honda et al. reported that serum MDA-LDL levels were significantly lower in patients with CKD stage 5 than in those with CKD stage 2-3 or stage 4 [20], it is possible that a decreased eGFR obscures the association between serum oxLDL levels and

severity of albuminuria. However, this possibility is unlikely because serum MDA-LDL levels had a significant positive association with the AER but no significant association was observed between serum MDA-LDL levels and the eGFR in the current study.

In the current study, the MDA-LDL/LDL was significantly correlated with both albuminuria and eGFR in bivariate analyses. However, those associations did not persist after adjustment for confounding factors. To our knowledge, this is the first investigation of the association between MDA-LDL/LDL and albuminuria or eGFR. It was shown that the oxLDL-to-LDL-C ratio or MDA-LDL/LDL was a superior predictor of coronary artery disease compared with the serum MDA-LDL level in cross-sectional studies [5, 21] and a prospective study [22]. Both increased urinary albumin levels and decreased eGFRs were reportedly independent predictors of CVD in type 2 diabetes [23-25]. In the current study, not serum MDA-LDL levels but the MDA-LDL/LDL was an independent predictor of CVD after adjustment for confounding factors including AER and eGFR or complicating diabetic kidney disease.

It was shown that serum oxLDL or MDA-LDL levels had positive associations with serum TG and

LDL-C levels [26-28] and negative associations with serum HDL-C levels [28]. Dyslipidemia in CKD is characterized by elevated serum TG levels and increased proportions of small dense LDL particles and decreased serum HDL-C [29]. Serum LDL-C values are increased according to the severity of albuminuria [30]. The current results showed that serum ln TG and LDL-C were significantly and positively correlated with serum MDA-LDL levels in bivariate analyses. MDA-LDL/LDL had a significantly positive correlation with ln TG and a negative correlation with LDL-C. Serum HDL-C levels had a significantly negative correlation with both serum MDA-LDL and the MDA-LDL/LDL. Serum ln TG and LDL-C but not serum ln HDL-C, ln AER, and eGFR were independent determinants of serum MDA-LDL levels and MDA-LDL/LDL in multiple linear regression analyses. The studies in which serum TG and/or LDL-C were increased according to the severity of albuminuria showed significantly increased serum oxLDL levels in macroalbuminuria in type 2 diabetes [13, 18]. On the other hand, serum LDL-C values seemed not to be different among categories of albuminuria in the study that showed no association between serum oxLDL levels and severity of albuminuria [19]. Consequently, increased serum MDA-LDL and MDA-LDL/LDL accompanied by increased

AERs or decreased eGFRs probably result from dyslipidemia associated with diabetic kidney disease.

There is limited evidence of the association between MDA-LDL or oxLDL and CKD in nondiabetic study participants. Kuchta and colleagues reported that plasma oxLDL levels were significantly increased in all stages (stage 3, stage 4, stage 5, hemodialysis and peritoneal dialysis) of CKD including that in patients with diabetes in addition to CKD in comparison with healthy study participants [31]. They also showed that mean plasma TG levels in all CKD groups were higher than that in healthy individuals [31]. However, this study did not analyze the association between circulating oxLDL levels and TG levels with bivariate or multivariate analysis so it remains unclear whether the association between circulating oxLDL levels and CKD stages is independent of dyslipidemia.

Although serum levels of TG and LDL-C were the only independent determinants of both serum MDA-LDL levels and MDA-LDL/LDL, these lipid parameters could only contribute less than 50% to the value of MDA-LDL-related variables (Table 5). The logistic regression analyses showed that the MDA-LDL/LDL was an independent determinant of the presence of CVD after adjustments for age, sex, duration of

diabetes, hypertension, smoking, dyslipidemia and diabetic kidney disease. Therefore, measuring serum MDA-LDL levels in addition to serum lipid levels could provide further information when assessing cardiovascular risk in patients with advanced diabetic kidney disease.

Several limitations of this study should be addressed. First, it was retrospective and cross-sectional, which does not allow us to derive any cause–effect relationship. Although both AER and eGFR were not independent determinants of MDA-LDL-related variables in this study, the possibility that increased MDA-LDL or MDA-LDL/LDL promotes albuminuria and/or an eGFR decline is not excluded. Moreover, there was a small number of participants with macroalbuminuria or an eGFR of less than 30 mL/min/1.73 m^2. Therefore, we might have underestimated the association between MDA-LDL or MDA-LDL/LDL and albuminuria or eGFR in the multivariate analyses. Serum LDL-C and ln TG were independent determinants of MDA-LDL-related variables. Seventy percent of the participants with CVD were taking lipid-lowering drugs in contrast to 34% of those without CVD. Thus, taking lipid-lowering drugs could obscure the association between the MDA-LDL-related variables and the risk of CVD (data not shown). Second, most of our patients had been

hospitalized for poor glycemic control. These issues could have introduced selection bias. Our results should be confirmed in prospective studies.

In conclusion, increased serum MDA-LDL levels and MDA-LDL/LDL in type 2 diabetic patients with albuminuria or decreased eGFR possibly results from dyslipidemia associated with diabetic kidney disease.

Author's contributions

SF designed the study, analyzed the data, and wrote the manuscript. HSu and KF designed the study and wrote the manuscript. KK, HI, SY, YS, MS, NY, and HSh contributed to the discussion and reviewed the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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Table 1. Clinical characteristics of participants

n	402		
Female, *n* (%)	159 (40)	Antidiabetic drugs, *n* (%)	
Age (year)	57 ± 13	Sulfonylureas	164 (41)
Duration of diabetes (year)	9.0 (3.0 – 16.0)	Metformin	164 (41)
Body mass index (kg/m^2)	27.0 ± 5.4	Thiazolidinedione	27 (7)
Hypertension*, *n* (%)	278 (69)	Glinides	11 (3)
Systolic blood pressure (mmHg)	134 ± 20	α-Glucosidase inhibitors	59 (15)
Diastolic blood pressure (mmHg)	78 ± 13	DPP-4 inhibitors	190 (47)
Current smoker, *n* (%)	107 (27)	GLP-1 receptor agonists	15 (4)
Cardiovascular disease, *n* (%)	84 (21)	SGLT-2 inhibitors	4 (1)
Estimated GFR (mL/min/1.73m^2)	86.6 ± 29.5	Insulin	133 (33)
Albumin excretion rate (mg/day)	13.8 (6.2 - 47.7)	Antihypertensive drugs, *n* (%)	
Fasting plasma glucose (mmol/L)	9.4 ± 2.7	Renin-angiotensin system inhibitors	154 (38)
HbA1c (%)	9.9 ± 1.9	Calcium channel blockers	138 (34)
C-reactive protein (mg/dL)	0.80 (0.03 – 0.23)	Diuretics	49 (12)
Total cholesterol (mmol/L)	5.0 ± 1.2	β-blockers	35 (8)
Triglycerides (mmol/L)	1.6 (1.1 – 2.2)	Lipid lowering drugs	
HDL cholesterol (mmol/L)	1.09 (0.92 – 1.30)	Statins	152 (38)
LDL cholesterol (mmol/L)	3.1 ± 1.0	Fibrates	11 (3)
MDA-LDL (U/L)	107 ± 50	Ezetimibe	7 (2)
MDA-LDL/LDL ([U/L] / [mmol/L])	36 ± 14	Eicosapentaenoic acid	12 (3)

Data are mean ± SD or median (interquartile range). Hypertension: systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 or taking antihypertensive drugs. DPP-4, dipeptidyl peptidase-4; GFR, glomerular filtration rate; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDA-LDL, malondialdehyde-modified low-density lipoprotein; MDA-LDL/LDL, MDA-LDL-to-LDL cholesterol ratio; SGLT-2, sodium-glucose cotranspoter-2

Table 2. Associations between albumin excretion rate and lipid parameters

	Albumin excretion rate（mg/day）			*P*	*P for trend*
	< 30	30 - < 300	300 -		
n	266	97	39		
MDA-LDL (U/L)	103 ± 44	109 ± 54	135 ± 72*††	0.006	0.020
MDA-LDL/LDL ([U/L] / [mmol/L])	35 ± 13	37 ± 14	40 ± 15*	0.008	0.003
Total cholesterol (mmol/L)	4.9 ± 1.0	4.9 ± 1.2	5.9 ± 2.0*†	0.012	0.298
Triglycerides (mmol/L)	1.5 (1.1 – 2.1)	1.7 (1.1 – 2.6)	2.3 (1.7 – 3.5)***††	< 0.001	< 0.001
HDL cholesterol (mmol/L)	1.10 (0.93 – 1.37)	1.07 (0.91 – 1.24)	1.09 (0.89 – 1.24)	0.124	0.044
LDL cholesterol (mmol/L)	3.0 ± 0.9	3.0 ± 1.0	3.4 ± 1.5	0.306	0.952

Data are mean ± SD or median (interquartile range). **P* <0.05 and ****P* <0.001 vs. albumin excretion rate of <30. †*P* <0.05 and ††*P* <0.01 vs. albumin excretion rate of 30 - <300. HDL; high-density lipoprotein; LDL, low-density lipoprotein; MDA-LDL, malondialdehyde-modified low-density lipoprotein; MDA-LDL/LDL, MDA-LDL-to-LDL cholesterol ratio.

Table 3. Associations between estimated GFR and serum lipid parameters

	Estimated GFR (mL/min/1.73m^2)				*P*	*P for trend*
	90 -	< 90 - 60	< 60 - 30	< 30		
n	172	158	61	11		
MDA-LDL (U/L)	108 ± 50	107 ± 49	100 ± 45	141 ± 90	0.321	0.527
MDA-LDL/LDL ([U/L] / [mmol/L])	34 ± 13	36 ± 13	38 ± 12	51 ± 28	0.013	0.002
Total cholesterol (mmol/L)	5.2 ± 1.1	5.0 ± 1.3	4.6 ± 1.2*	5.2 ± 1.5	0.002	< 0.001
Triglycerides (mmol/L)	1.6 (1.1 – 2.2)	1.5 (1.1 – 2.1)	1.7 (1.3 – 2.4)	2.3 (2.0 – 2.6)†	0.019	0.424
HDL cholesterol (mmol/L)	1.10 (0.96 – 1.35)	1.13 (0.97 – 1.33)	0.98 (0.85 – 1.13)**††	0.89 (0.74 – 1.08)	< 0.001	0.001
LDL cholesterol (mmol/L)	3.2 ± 0.9	3.1 ± 1.0	2.7 ± 1.0***†	2.9 ± 1.1	< 0.001	< 0.001

Data are mean ± SD or median (interquartile range). *P <0.05, **P <0.01 and **P <0.001 vs. eGFR of 90 -, †P <0.05 and ††P <0.01 vs. eGFR of <90 – 60. HDL; high-density lipoprotein; LDL, low-density lipoprotein; MDA-LDL, malondialdehyde-modified low-density lipoprotein; MDA-LDL/LDL, MDA-LDL-to-LDL cholesterol ratio

Table 4. Correlations between MDA-LDL-related variables and clinical parameters

	MDA-LDL		MDA-LDL/LDL	
	r	*P*	*r*	*P*
Age	-0.221	<0.001	-0.049	0.326
Systolic blood pressure	0.047	0.350	0.017	0.734
Diastolic blood pressure	0.187	< 0.001	0.088	0.077
Hypertension [a)]	-0.007	0.885	0.150	0.003
Cardiovascular disease [a)]	-0.095	0.056	0.178	< 0.001
Fasting plasma glucose	0.137	0.006	0.074	0.136
HbA1c	0.180	<0.001	0.032	0.528
Estimated GFR	-0.001	0.989	-0.138	0.005
ln Albumin excretion rate	0.200	< 0.001	0.157	0.002
Statins [a)]	-0.188	< 0.001	0.199	< 0.001
Fibrates [a)]	-0.028	0.578	-0.028	0.579
ln Triglycerides	0.486	< 0.001	0.504	< 0.001
ln HDL cholesterol	-0.158	0.001	-0.259	< 0.001
LDL cholesterol	0.547	< 0.001	-0.182	< 0.001

Parametric values are tested by Pearson's correlation. a) Non-parametric values are tested by Spearman's correlation. GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ln HDL cholesterol, ln-transformed high-density lipoprotein cholesterol; ln triglycerides, ln-transformed triglycerides; MDA-LDL, malondialdehyde-modified low-density lipoprotein; MDA-LDL/LDL, MDA-LDL-to-LDL cholesterol ratio

Table 5. Multivariate association of MDA-LDL-related variables with clinical parameters

	MDA-LDL (Adjusted R^2 = 0.451)			MDA-LDL/LDL (Adjusted R^2 = 0.332)		
Covariates	β	Standard Error	*P*	β	Standard Error	*P*
Age	-0.107	0.192	0.579	-0.054	0.058	0.346
Sex	3.243	4.224	0.443	0.817	1.269	0.520
Cardiovascular disease	1.531	5.136	0.766	0.933	1.543	0.546
Current smoker	-6.638	4.734	0.162	-2.356	1.422	0.098
BMI	-0.596	0.408	0.145	-0.236	0.123	0.055
Hypertension	4.400	5.245	0.402	1.204	1.576	0.445
Fasting plasma glucose	-1.261	0.845	0.136	-0.420	0.254	0.099
HbA1c	2.063	1.187	0.083	0.566	0.357	0.113
eGFR	-0.082	0.082	0.317	-0.027	0.025	0.266
ln AER	0.291	1.228	0.813	-0.019	0.369	0.958
ln Triglycerides	38.185	3.556	< 0.001	15.286	1.390	< 0.001
ln HDL cholesterol	1.792	9.343	0.848	1.021	2.807	0.716
LDL cholesterol	24.518	1.928	< 0.001	-4.084	0.692	<0.001
Taking statins and/or ezetimibe	-2.823	4.642	0.543	-1.258	1.395	0.368
Taking fibrates and/or EPA	-4.887	8.311	0.557	-1.766	2.497	0.480
Taking renin-angiotensin system inhibitors	1.463	4.879	0.764	1.870	1.466	0.203

BMI, body mass index; eGFR, estimated glomerular filtration rate; ln AER, ln-transformed albumin excretion rate; ln Triglycerides, ln-transformed triglycerides; ln HDL, ln-transformed high-density lipoprotein; LDL, low-density lipoprotein; EPA, eicosapentaenoic acid; MDA-LDL, malondialdehyde-modified-low density lipoprotein; MDA-LDL/LDL, MDA-LDL-to-LDL cholesterol ratio

Supplemental Table. Logistic regression models for variables associated with the presence of cardiovascular disease

	Model 1				Model 2			
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.03 (1.01 – 1.06)	0.010	1.04 (1.01 – 1.07)	0.003	1.04 (1.02 – 1.07)	0.001	1.04 (1.02 – 1.07)	0.000
Male sex	0.92 (0.46 – 1.82)	0.808	0.85 (0.43 – 1.70)	0.651	0.90 (0.46 – 1.78)	0.769	0.83 (0.42 – 1.63)	0.580
Duration of diabetes	1.03 (1.00 – 1.06)	0.039	1.03 (1.00 – 1.06)	0.024	1.03 (1.00 – 1.06)	0.032	1.03 (1.00 – 1.07)	0.022
Hypertension	4.13 (1.81 – 9.43)	0.001	3.92 (1.71 – 8.97)	0.001	3.79 (1.65 – 8.70)	0.002	3.57 (1.55 – 8.19)	0.003
Smoking	1.99 (0.98 – 4.07)	0.058	2.09 (1.03 – 4.25)	0.042	1.93 (0.96 – 3.89)	0.065	2.04 (1.01 – 4.13)	0.046
Dyslipidemia	1.52 (0.69 – 3.32)	0.292	1.33 (0.60 – 2.92)	0.484	1.65 (0.76 – 3.60)	0.208	1.38 (0.63 – 3.05)	0.419
eGFR	0.99 (0.98 – 1.00)	0.085	0.99 (0.98 – 1.00)	0.178	–	–	–	–
AER	1.00 (1.00 – 1.00)	0.596	1.00 (0.90 – 1.00)	0.439	–	–	–	–
Diabetic kidney disease	–	–	–	–	1.82 (1.06 – 3.15)	0.031	1.64 (0.95 – 2.85)	0.077
MDA-LDL	1.00 (0.99 – 1.00)	0.466	–	–	1.00 (0.99 – 1.00)	0.349	–	–
MDA-LDL/LDL	–	–	1.02 (1.00 – 1.04)	0.032	–	–	1.02 (1.00 – 1.04)	0.035

Hypertension, systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 and/or taking antihypertensive drugs; Smoking, having current and/or ever smoking history; Dyslipidemia, serum triglycerides levels ≥ 1.7 mmol/L and/or serum high-density lipoporotein cholesterol levels < 1.03 mmol/L and/or low-density lipoprotein cholesterol levels ≥ 3.62 mmol/L and/or taking lipid-lowering drugs; eGFR, estimated glomerular filtration rate; AER, albumin excretion rate; Diabetic kidney disease, eGFR < 60 and/or AER ≥ 30 mg/day; MDA-LDL, malondialdehyde-modified-low density lipoprotein; MDA-LDL/LDL, MDA-LDL-to-LDL cholesterol ratio; OR, odds ratio; CI, confidence interval