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ORIGINAL ARTICLE

Evaluation of Malondialdehyde Low-Density Lipoprotein Stratified by Low-Density Lipoprotein Cholesterol

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SUMMARY

Background: **Malondialdehyde low-density lipoprotein (MDA-LDL) is a major form of oxidized LDL and considered to be more atherogenic than LDL. Information on major determinants of MDA-LDL and their association in subjects who are not under treatment for diabetes mellitus and dyslipidemia is limited.**
Methods: **This study included 778 Japanese subjects who were not taking medication for diabetes mellitus and dyslipidemia. All subjects underwent an annual health examination that included MDA-LDL analysis. Study subjects were divided into four groups according to mean values of LDL-C and MDA-LDL, and the metabolic profile was compared.**
Results: **LDL cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were mainly associated with MDA-LDL. When subjects were stratified based on LDL-C levels, small dense LDL-C and MDA-LDL levels increased as LDL-C levels increased. Comparison of the characteristics of study subjects in the same LDL-C level group revealed that subjects with high MDA-LDL showed high metabolic risk in all LDL-C groups, particularly notable in the group with LDL-C levels < 120 mg/dL.**
Conclusions: **Our data indicated that high LDL-C and low HDL-C levels were independently associated with high MDA-LDL. To prevent high MDA-LDL, it is important to lower LDL-C level as well as to increase HDL-C even in subjects with low LDL-C level by lifestyle modification.**
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KEY WORDS

MDA-LDL, LDL-C, HDL-C, metabolic syndrome, annual health examination

INTRODUCTION

Low-density lipoprotein cholesterol (LDL-C) comprises multiple distinct subclasses of particles that differ in size, density, physicochemical composition, metabolic behavior, and atherogenicity [1]. Small, dense LDL (sdLDL) particles are more likely to form oxidized LDL, are less readily cleared, and are highly atherogenic [2]. Increase in reactive oxygen species, which often occurs with various cardiovascular risk factors, such as dyslipidemia, hypertension, and diabetes mellitus, leads to the formation of oxidized LDL [3]. Modified LDL, including oxidized LDL, is taken up by scavenger receptors, which do not bind to native LDL. Scavenger re-

ceptor-mediated uptake of oxidized LDL induces foam cell formation *in vitro*, leading to the development of atherosclerotic lesions [4]. Malondialdehyde LDL (MDA-LDL) is considered a major form of oxidized LDL; however, the origin of this particle is not well understood. MDA-LDL plays a key role in the progression of atherosclerosis [2]. Associations of increased serum MDA-LDL levels with coronary artery disease (CAD) [5-7] or coronary artery calcification [8] are also documented. Serum MDA-LDL levels have also been positively correlated with carotid intima-media thickness [6, 9]. Although most of the studies were conducted in patients with type 1 diabetes mellitus [2], CAD, [5-7] undergoing hemodialysis [8], and type 2 diabetes mellitus [10], there is, thus far, no specific treatment to reduce MDA-LDL levels.

In the coronary artery risk development in young adults (CARDIA) study, higher oxidized LDL levels were associated with increased incidence of metabolic syndrome as well as its components of abdominal obesity, hyperglycemia, and hypertriglyceridemia [10]. During a 5-year follow up, subjects in the highest quartile of oxidized LDL level had a three-fold risk for the development of metabolic syndrome compared with those in the lowest quartile [10].

Therefore, an evaluation of serum MDA-LDL levels is an important step for protection from atherosclerotic events. The relationship of MDA-LDL and LDL-C levels with lipid markers, metabolic risk, and characteristics of subjects with different lipid levels is not well examined.

This study aimed to clarify the characteristics of subjects stratified by LDL-C and MDA-LDL levels and their association in subjects not receiving treatment for dyslipidemia and diabetes mellitus.

MATERIALS AND METHODS

Subjects

A total of 944 subjects underwent annual health examinations, including MDA-LDL analysis, at the Health Evaluation and Promotion Center, Tokai University Hachioji Hospital, between April 2011 and March 2014. After excluding 166 subjects who were taking medication for diabetes mellitus and dyslipidemia, 778 subjects (502 men and 276 women) were ultimately included in this study. Medical histories were obtained using self-administered questionnaires and interviews conducted by nurses.

Measurements

Waist circumference (WC) was measured at the level of the umbilicus while the subject was standing and during slight expiration. Blood pressure (BP) was measured on the upper right arm using an automatic BP monitor (TM-2655P; A&D, Tokyo, Japan) while the subject was seated. Blood samples were collected in tubes coated with heparin early in the morning after an overnight fast. Fasting plasma glucose was measured using hexokinase G-6-PDH method with a Wako L-type Glu 2 kit (Wako Pure Chemicals, Osaka, Japan). Fasting immunoreactive insulin (FIRI) levels were measured using a fluorescence enzyme immunoassay (ST AIA-PACK IRI; Toso, Tokyo, Japan). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: fasting plasma glucose (FPG) (mg/dL) x FIRI (μU/mL)/405 [11]. LDL-C was calculated using the Friedewald formula [12]. High density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels were measured using visible spectrophotometry (Determiner L HDL-C, and Determiner L TG II, respectively; Kyowa Medex, Tokyo, Japan). SdLDL-C levels were measured using a homogeneous method (sdLDL-Ex; DENKA SEIKEN Co., Tokyo, Japan). MDA-LDL levels were measured via enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies specific to MDA-LDL (ML25) and apolipoprotein B (AB16) (Sekisui Medical, Tokyo, Japan). It is well known that the combination of ML25 and AB16 antibodies can accurately detect MDA-LDL [13].

Verbal consent for the analytical use of anonymized health records was obtained from all subjects. The study protocol was approved by the institutional ethics committee of the Tokai University School of Medicine.

Statistical analysis

Scheffe's multiple comparisons test was used to compare mean values across more than two groups. MDA-LDL values in the same LDL-C level after stratifying subjects according to HDL-C levels were compared. Multiple linear regression analysis was performed to identify significant determinants of MDA-LDL. Body mass index (BMI), WC, LDL-C, HDL-C, TG, and FPG levels and systolic and diastolic BP were the used as independent variables. Metabolic profile was compared after subjects were divided into four groups based on LDL-C levels and the subjects with the same LDL-C group were further divided into three, according to tertile of MDA-LDL levels. A stepwise procedure was used to select variables for the multiple linear regression analysis. Statistical analyses were performed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA). All p-values were two-tailed, and a p-value < 0.05 was considered statistically significant.

RESULTS

Table 1 lists the subjects' characteristics. There were 276 (35.5%) women in this study. The mean sdLDL-C and MDA-LDL levels were 37.7 mg/dL and 140.1 U/L, respectively.

Table 2 shows the multiple liner regression analysis for determinants of MDA-LDL. Of the following variables, BMI, WC, FPG, HDL-C, and TG levels, and systolic and diastolic BP, four variables (LDL-C, HDL-C, diastolic BP and TG) were selected by stepwise procedure.

Table 1. Characteristics of study subjects.

Age (years)	**58.0 ± 12.0**
BMI (kg/m^2)	**23.2 ± 3.2**
Waist circumference (cm)	**82.7 ± 8.9**
Systolic BP (mmHg)	**122.1 ± 17.3**
Diastolic BP (mmHg)	**77.1 ± 12.8**
FPG (mg/dL)	**101.6 ± 14.0**
FIRI (μIU/mL)	**5.95 ± 4.35**
HOMAR-IR	**1.54 ± 1.41**
TG (mg/dL)	**111.5 ± 74.5**
HDL-C (mg/dL)	**65.3 ± 17.3**
LDL-C (mg/dL)	**124.8 ± 30.5**
SdLDL-C (mg/dL)	**37.7 ± 15.5**
MDA-LDL (U/L)	**140.1 ± 43.7**

Variables are given as means ± standard deviations.
BMI - body mass index, BP - blood pressure, FPG - fasting plasma glucose, FIRI - fasting immunoreactive insulin, HOMA-IR - homeostasis model assessment of insulin resistance, TG - triglyceride, HDL-C - high-density lipoprotein cholesterol, LDL-C - low-density lipoprotein cholesterol calculated by Friedewald formula, sdLDL-C - small, dense low-density lipoprotein cholesterol, MDA-LDL - malondialdehyde low-density lipoprotein.

Table 2. Multiple liner regression analysis for MDA-LDL.

	MDA-LDL			
	RC	**SRC**	***t***	**p**
LDL-C	**0.872**	**0.609**	**24.2**	**< 0.0001**
HDL-C	**-0.604**	**-0.239**	**-8.52**	**< 0.0001**
Diastolic BP	**0.464**	**0.136**	**5.32**	**< 0.0001**
TG	**0.079**	**0.135**	**4.75**	**< 0.0001**

Variable selection was made using a stepwise procedure.
MDA-LDL - malondialdehyde low-density lipoprotein, RC - regression coefficient, SRC - standardized regression coefficient, LDL-C - low-density lipoprotein cholesterol calculated by Friedewald formula, HDL-C - high-density lipoprotein cholesterol, BP - blood pressure, TG - triglyceride.

Standardized regression coefficients were higher for LDL-C and HDL-C levels than for the other selected variables. Thus, high LDL-C and low HDL-C levels were mainly associated with high MDA-LDL levels.

Figure 1 presents a bar graph of MDA-LDL levels when subjects were stratified according to LDL-C levels in four groups (LDL-C levels, 0 - < 120, 120 - < 140, 140 - < 160, and ≥ 160 mg/dL) and according to HDL-C in three groups by tertile: (HDL-C levels, ≥ 70, 55 - < 70, and < 55 mg/dL). Numbers on the bars represent the mean values of MDA-LDL and n indicates the number of subjects of each group. MDA-LDL levels increased as HDL-C levels decreased in all LDL-C groups.

Table 3 shows the characteristics of study subjects stratified by LDL-C levels. SdLDL-C and MDA-LDL levels increased as LDL-C levels increased. However, most of the other parameters, including HDL-C levels, were not different across LDL-C levels.

Table 4 shows the characteristics of study subjects after stratification by LDL-C and MDA-LDL levels. To clarify the characteristics of study subjects with the same LDL-C level, study subjects were divided into four groups. In each group, subjects were further divided into three, according to tertile of MDA-LDL levels. The table shows the MDL-LDL range and numbers of subjects in each group. In subjects with an LDL-C level of ≥ 160 mg/dL, as MDA-LDL levels increased, BMI and TG, LDL-C, and sdLDL-C levels increased, while HDL-C levels decreased. In subjects with LDL-C levels of 140 - < 160 mg/dL, BMI, WC, diastolic BP, and TG, LDL-C, and sdLDL-C levels increased and HDL-C levels decreased as MDA-LDL levels increased. In subjects with LDL-C of 120 - < 140 mg/dL, WC, systolic and diastolic BP, and FPG, TG, LDL-C, and sdLDL-C levels increased and HDL-C levels decreased as MDA-LDL levels increased. In subjects with LDL-C levels < 120 mg/dL, BMI, WC, systolic and diastolic BP, and FPG, TG, LDL-C, and sdLDL-C levels increased and HDL-C levels decreased as MDA-LDL levels increased. Overall, subjects with high MDA-LDL levels showed high metabolic risk in all LDL-C groups; this was particularly notable in the group with the lowest LDL-C levels (LDL-C level of < 120 mg/dL).

DISCUSSION

We showed that high LDL-C and low HDL-C levels were associated with high MDA-LDL levels. Comparison of the characteristics of study subjects with the same LDL-C levels revealed that those with high MDA-LDL levels showed high metabolic risk in all LDL-C groups. This was particularly notable in the group with LDL-C < 120 mg/dL.

The mean MDA-LDL levels in healthy Japanese men [14,15] were almost compatible with our result. Among Japanese subjects, wide ranges of MDA-LDL in subjects with various baseline characters were reported [8, 9,14-16]. Since a comparable assay was used, the reason for the difference in absolute MDA-LDL levels is not clear. Relatively small number of subjects [16], treatments for dyslipidemia and diabetes mellitus [8,9, 16], and hemodialysis [8] could account for the difference.

The hypothesis which assigns a crucial role to oxidized LDL in the initiation and progress of atherosclerosis is still debated. However, several studies have reported significant elevations of circulating oxidized LDL levels not only in patients with CAD [5-7] but also in patients with diabetes mellitus [17], insulin resistance [18], and

Table 3. Characteristics of study subjects stratified by LDL-C.

LDL-C (mg/dL)	< 120 (n = 333)	120 - < 140 (n = 212)	140 - < 160 (n = 135)	≥ 160 (n = 98)
BMI (kg/m^2)	22.6 ± 3.2	23.6 ± 3.2 *	23.6 ± 3.5	23.6 ± 2.9
Systolic BP (mmHg)	120.7 ± 16.9	122.6 ± 17.4	122.2 ± 17.4	125.4 ± 18.3
Diastolic BP (mmHg)	77.1 ± 12.9	77.1 ± 12.9	75.8 ± 12.1	78.6 ± 13.0
FPG (mg/dL)	100.3 ± 12.5	101.7 ± 13.0	102.7 ± 13.7	104.2 ± 19.9
FIRI (μIU/mL)	5.66 ± 4.43	5.98 ± 4.35	6.00 ± 3.86	6.78 ± 4.68
HOMA-IR	1.47 ± 1.59	1.53 ± 1.27	1.55 ± 1.08	1.79 ± 1.43
TG (mg/dL)	110.3 ± 92.8	109.6 ± 55.8	113.7 ±64.1	116.1 ± 49.6
HDL-C (mg/dlL)	66.6 ± 17.6	64.8 ± 18.4	63.8 ± 16.0	64.0 ± 15.3
SdLDL-C (mg/dL)	29.6 ± 12.0	38.6 ± 13.0 **	44.2 ± 14.7 **, ##	53.9 ± 14.4 **, ##, $$
MDA-LDL (U/L)	115.4 ± 32.3	145.3 ± 34.2 *	157.7 ± 39.6 **, ##	188.5 ± 45.7 **, ##, $$

Variables are given as means ± standard deviations.
LDL-C - low-density lipoprotein cholesterol calculated by Friedewald formula. BMI - body mass index, BP - blood pressure, FPG - fasting plasma glucose, FIRI - fasting immunoreactive insulin, HOMA-IR - homeostasis model assessment of insulin resistance TG - triglyceride, HDL-C - high-density lipoprotein cholesterol, sdLDL-C - small, dense low-density lipoprotein cholesterol, MDA-LDL - malondialdehyde low-density lipoprotein. ** p < 0.01, * p < 0.05 (LDL-C < 120 vs. 120 - < 140 mg/dL, LDL-C < 120 vs. 140 - < 160 mg/dL, LDL-C < 120 vs. ≥ 160 mg/dL), ## p < 0.01, # p < 0.05 (LDL-C < 120 vs. 140 - < 160 mg/dL, LDL-C < 120 vs. ≥ 160 mg/dL), $$ p < 0.01, $ p < 0.05 (LDL-C 140 - < 160 vs. ≥ 160 mg/dL) by Scheffe's multiple comparison test.

Table 4. Characteristics of study subjects stratified by LDL-C and MDA-LDL.

(a) LDL-C ≥ 160 (mg/dL)

MDA-LDL (U/L)	< 160 (①) (n = 31)	160 - < 200 (②) (n = 31)	≥ 200 (③) (n = 36)
BMI (kg/m^2)	22.7 ± 2.6	23.5 ± 2.7	24.6 ± 3.2 *
Waist circumference (cm)	81.9 ± 9.1	83.5 ± 6.5	85.2 ± 6.7
Systolic BP (mmHg)	123.5 ± 18.7	129.8 ± 20.3	123.3 ±15.7
Diastolic BP (mmHg)	76.0 ± 11.8	79.7 ± 13.7	79.8 ± 13.3
FPG (mg/dL)	104.5 ± 29.6	104.0 ± 13.5	104.2 ± 13.7
FIRI (μIU/mL)	5.96 ± 4.67	6.72 ± 4.41	7.54 ± 4.91
HOMAR-IR	1.57 ± 1.39	1.79 ± 1.43	1.98 ± 1.48
TG (mg/dL)	94.7 ± 34.6	111.2 ± 42.2	138.7 ± 57.6 **
HDL-C (mg/dlL)	72.3 ± 17.1	66.2 ± 13.2 *	55.0 ±10.2 **, ##
LDL-C (mg/dL)	171.7 ± 13.2	175.9 ± 11.7	180.3 ± 18.3 *

(b) LDL-C 140 - < 160 (mg/dL)

MDA-LDL (U/L)	< 140 (①) (n = 44)	140 - < 170 (②) (n = 43)	≥ 170 (③) (n = 48)
BMI (kg/m^2)	23.1 ± 3.9	22.8 ± 3.0	24.7 ± 3.2 #
Waist circumference (cm)	81.2 ± 10.9	82.4 ± 8.1	86.9 ± 7.6 *
Systolic BP (mmHg)	119.1 ± 19.3	123.3 ± 18.0	124.0 ± 14.9
Diastolic BP (mmHg)	71.3 ± 11.8	75.0 ± 11.7	80.6 ± 11.2 **
FPG (mg/dL)	103.3 ± 17.7	99.8 ± 9.5	104.8 ± 12.5
FIRI (μIU/mL)	5.48 ± 4.33	5.67 ± 2.92	6.78 ± 4.10
HOMAR-IR	1.43 ± 1.23	1.41 ± 0.79	1.80 ± 1.15
TG (mg/dL)	92.5 ± 29.5	105.5 ± 43.5	140.5 ± 89.5 **, #
HDL-C (mg/dlL)	70.8 ± 14.6	64.5 ± 13.4	56.8 ± 16.8 **
LDL-C (mg/dL)	148.4 ± 5.3	147.7 ± 5.5	148.8 ± 5.5

Table 4. Characteristics of study subjects stratified by LDL-C and MDA-LDL (continue).

(c) LDL-C 120- < 140 (mg/dL)

MDA-LDL (U/L)	< 130 (①) (n = 74)	130 - < 160 (②) (n = 67)	≥ 160 (③) (n = 71)
BMI (kg/m^2)	22.9 ± 2.9	24.1 ± 3.8	24.0 ± 2.8
Waist circumference (cm)	81.2 ± 8.6	84.6 ± 9.2	85.5 ± 7.6 **
Systolic BP (mmHg)	117.6 ± 18.7	124.2 ± 14.2	126.2 ± 17.8 *
Diastolic BP (mmHg)	74.4 ± 13.9	77.4 ± 12.1	79.7 ± 12.2 *
FPG (mg/dL)	98.6 ± 9.2	101.5 ± 10.2	105.0 ± 17.3 *
FIRI (μIU/mL)	5.26 ± 3.32	6.53 ± 6.07	6.21 ± 3.16
HOMAR-IR	1.31 ± 0.95	1.69 ± 1.80	1.62 ± 0.89
TG (mg/dL)	87.4 ± 35.0	109.7 ± 50.7 *	132.8 ± 67.9 $^{**, \#}$
HDL-C (mg/dlL)	73.1 ± 19.1	62.2 ± 18.7 **	58.6 ± 13.7 **
LDL-C (mg/dL)	127.6 ± 5.4	130.0 ± 5.8	130.3 ± 6.1 *

(d) LDL-C < 120 (mg/dL)

MDA-LDL (U/L)	< 100 (①) (n = 121)	100 - < 125 (②) (n = 96)	≥ 125 (③) (n = 116)
BMI (kg/m^2)	21.6 ± 2.9	22.7 ± 3.4 *	23.8 ± 2.9 $^{**, \#}$
Waist circumference (cm)	78.3 ± 8.3	81.4 ± 9.8 *	84.5 ± 8.7 $^{**, \#}$
Systolic BP (mmHg)	116.1 ± 16.5	120.7 ±17.0	125.5 ± 16.0 **
Diastolic BP (mmHg)	74.1 ± 12.0	76.4 ± 12.9	80.8 ± 13.0 $^{**, \#}$
FPG (mg/dL)	97.0 ± 14.1	100.9 ± 11.2	103.1 ± 10.8 **
FIRI (μIU/mL)	4.50 ± 4.50	5.46 ± 3.90	7.04 ± 4.42 $^{**, \#}$
HOMAR-IR	1.17 ± 2.08	1.39 ± 1.05	1.84 ± 1.28 **
TG (mg/dL)	90.7 ± 64.8	103.0 ±92.4	137.0 ± 110.7 $^{**, \#}$
HDL-C (mg/dlL)	72.3 ± 17.1	69.1 ± 17.3	58.5 ± 15.3 $^{**, \#\#}$
LDL-C (mg/dL)	88.8 ± 19.2	98.0 ± 16.9 **	105.3 ± 12.3 $^{**, \#\#}$

Variables are given as means ± standard deviations. Subjects were stratified into four groups according to LDL-C and MDA-LDL. ①; upper tertile, ②; middle tertile, ③; lower tertile of MDA-LDL in each group.
LDL-C - low-density lipoprotein cholesterol calculated by Friedewald formula, MDA-LDL - malondialdehyde low-density lipoprotein, BMI - body mass index, BP - blood pressure, FPG - fasting plasma glucose, FIRI - fasting immunoreactive insulin, HOMA-IR - homeostasis model assessment of insulin resistance, TG - triglyceride, HDL-C - high-density lipoprotein cholesterol, sdLDL-C - small, dense low-density lipoprotein cholesterol, MDA-LDL - malondialdehyde low-density lipoprotein.
** p < 0.01, * p < 0.05 (① vs. ②, ① vs. ③), $^{\#\#}$ p < 0.01, $^{\#}$ p < 0.05 (② vs. ③) by Scheffe's multiple comparison test.

obesity [5,19,20], suggesting that a state of higher metabolic risk could lead to high MDA-LDL levels. On the other hand, another study suggested that high circulating oxidized LDL levels could be the earlier step in the development of atherosclerosis [10]. Oxidized LDL could induce endothelial cell apoptosis and impairment of vasodilatation responses [21] in individuals without apparent atherosclerosis. It appears that oxidized LDL-induced toxic effects are present in all stages of atherosclerosis from the beginning to the occurrence of acute thrombotic events [22]. These studies suggest that earlier detection of oxidized LDL in people who show no apparent atherosclerosis is important for protection from the development of atherosclerosis. Our results indicated that when subjects with similar LDL-C levels were compared, those with high MDA-LDL levels showed high metabolic risk even in the group with LDL-C of < 120 mg/dL. Because MDA-LDL levels were positively correlated with LDL-C levels [14], subjects with high LDL-C and MDA-LDL levels should be supervised by physicians, and statin therapy will lower both LDL-C and oxidized LDL levels [23]. On the contrary, the subjects with low LDL-C and high MDA-LDL levels who have not developed diabetes, dyslipidemia, metabolic syndrome, and CAD may be at a lesser risk. Although several studies indicated that an increased circulating oxidized LDL level was a strong predictor of cardiovascular events [24-26], measuring MDA-LDL levels has

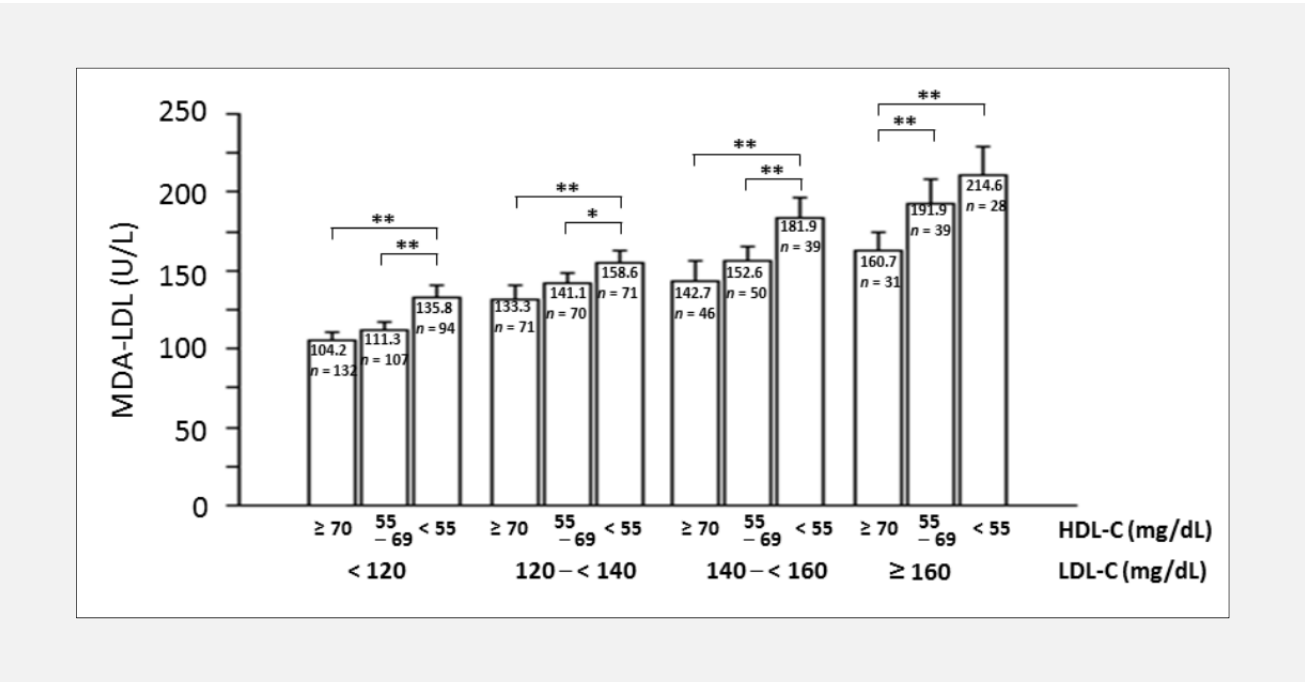

Figure 1. Bar graph of mean MDA-LDL values after stratifying subjects according to LDL-C and HDL-C levels.

**** p < 0.01, * p < 0.05 by Scheffe's multiple comparison test.**
MDA-LDL - malondialdehyde low-density lipoprotein, LDL-C - low-density lipoprotein cholesterol calculated using the Friedewald formula, HDL-C - high-density lipoprotein cholesterol.

not been widely used in daily clinical practice because of the limited availability of clinical data and methodological problems.

Our results also indicated that low HDL-C is associated with high MDA-LDL levels. This raises the possibility of intervention not only for subjects with high LDL-C and MDA-LDL levels who have already developed CAD but also for those with low LDL-C and high MDA-LDL levels who show no signs of atherosclerosis. Low HDL-C level is associated with the following characteristics: being overweight or obese [27-29], smoking [28,30], and weight loss [27]. Smoking cession [31] and regular aerobic exercise increases HDL-C levels [28,29,32,33]. Besides reversing cholesterol transport, the antioxidant properties of HDL may also play a major role in protection against the development of atherosclerosis [34]. Various mechanisms for the antioxidant properties of HDL have been reported. HDL carries apolipoprotein A-I (apo A-I), which is the main protein constituent of HDL, and apo A-I itself has antioxidant properties through a methionine residue [34]. Other apolipoproteins present on HDL, such as apo A-II, apo J, and apo E, which also have antioxidant properties. Moreover, HDL carries antioxidant enzymes that have the capacity to prevent lipid oxidation or degrade lipid hydroperoxides, such as serum paraoxonase arylesterase 1 (PON1), lecithin cholesterol acyltransferase (LCAT), and platelet activating factor acetylhydrolase (PAF-AH) [35]. As HDL-C levels decrease, the extent of MDA modification per one LDL particle increases [36]. To increase HDL levels, losing weight, quitting smoking, and doing regular aerobic exercise is therefore recommended, not only in subjects who have already developed CAD and are taking medication for high LDL-C levels but also for those with low LDL-C levels who have not developed diabetes mellitus, dyslipidemia, metabolic syndrome, or CAD.

It is reported that circulating oxidized LDL levels positively correlate with degrees of obesity [5,19,20]. Body weight reduction after gastric banding surgery decreased circulating oxidized LDL levels, which had been elevated in association with obesity [37]. The body weight reduction induced by dietary restriction also decreased plasma circulating oxidized LDL levels in postmenopausal women [38]. Importantly, in several studies [38], circulating oxidized LDL levels showed a much stronger association with the degree of obesity than did the LDL level. BMI was only a minor factor for MDA-LDL as judged by the standardized regression coefficient in this study. This is probably due to the characteristics of the study subjects. The proportion of our study subjects with BMI $\geq$ 25 was 26.5%. Among them, 33.5% of subjects showed HOMA-IR $\geq$ 2.5, which is considered to be insulin resistant (data not shown). A small portion of obese subjects accompanied by insulin resistance may contribute to these results.

A previous study reported epidemiological evidence linking circulating MDA-LDL to cardiovascular events in healthy subjects [39]. They concluded that the production of circulating MDA-LDL may be accelerated by insulin resistance, thus impairing endothelial function even in healthy young men [39]. Endothelial dysfunction is considered to be an early step in the development of atherosclerosis [40], thus lifestyle modification could prevent development of atherosclerosis via reducing production of MDA-LDL. Moreover, there is interventional evidence showing lifestyle modification can lower MDA-LDL [15,16]. After the 12-week Nordic walking training exercise, MDA-LDL decreased significantly [16]. Increased HDL-C level due to Nordic walking could contribute to decrease MDA-LDL in this case. Another recent study showed that 6 weeks intervention of switching Western food to the Japan Diet (more fish, soybeans and soy products, vegetables, seaweed, mushrooms and unrefined cereals, and less animal fat, meat and poultry with fat, sweets, desserts and snacks, and alcoholic drinks), resulted in significantly decreased circulating MDA-LDL concentrations [15]. Decreased TG due to Japan Diet intake resulted in increased HDL-C, which could contribute to decreased MDA-LDL level. The limitations of our study include its cross-sectional nature, which prevented the establishment of a causal relationship. The associations of MDA-LDL levels with HDL-C levels might have been confounded by factors such as diet, alcohol consumption, and exercise. The subjects of this study were middle-aged Japanese individuals, and it is possible that the relationship between MDA-LDL levels and clinical markers were affected by age and ethnicity. Moreover, detailed information regarding hypertension treatment was omitted from this study. Finally, our results were calculated from data of only a fraction of the subjects who underwent annual health examinations; therefore, they might not be generalizable to all Japanese subjects.

CONCLUSION

MDA-LDL was mainly determined by LDL-C and HDL-C levels in subjects who are not receiving treatment for diabetes mellitus and dyslipidemia. Subjects with high LDL-C and also those with low LDL-C levels should receive attention from a physician. The subjects with high LDL-C should be advised to reduce LDL-C. Rather than measuring MDA-LDL levels for everyone, we should be aware that subjects who have LDL-C < 120 mg/dL could have high MDA-LDL levels. The subjects with LDL-C <120 mg/dL and HDL-C < 40 mg/dL should be encouraged to improve their lifestyle and habits to increase their HDL levels for the prevention of increased MDA-LDL level.

Declaration of Interest:
There are no conflicts of interest to declare.

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