

BMJ Open

Increase in the Oxidized Low-Density Lipoprotein Level by Smoking and the Possible Inhibitory Effect of Statin Therapy in Patients with Cardiovascular Disease.

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-005455
Article Type:	Research
Date Submitted by the Author:	12-Apr-2014
Complete List of Authors:	Ogawa, Kazuo; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Tanaka, Toshikazu; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Nagoshi, Tomohisa; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Sekiyama, Hiroshi; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Arase, Satoshi; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Minai, Kosuke; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Ogawa, Takayuki; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Yoshimura, Michihiro; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Smoking and tobacco, Epidemiology
Keywords:	Coronary heart disease < CARDIOLOGY, Lipid disorders < DIABETES & ENDOCRINOLOGY, Cardiology < INTERNAL MEDICINE

SCHOLARONE™
Manuscripts

1
2
3
4
5
6 **Increase in the Oxidized Low-Density Lipoprotein Level by Smoking and the Possible**
7
8
9 **Inhibitory Effect of Statin Therapy in Patients with Cardiovascular Disease.**
10

11
12
13
14
15 Kazuo Ogawa, MD. ; Toshikazu Tanaka, MD. PhD. ; Tomohisa Nagoshi, MD. PhD.;

16
17
18 Hiroshi Sekiyama, MD. PhD. ; Satoshi Arase, MD. ; Kosuke Minai, MD. PhD. ;

19
20
21 Takayuki Ogawa, MD. PhD and Michihiro Yoshimura, MD. PhD.

22
23
24 Division of Cardiology, Department of Internal Medicine,

25
26
27 The Jikei University School of Medicine, Tokyo, Japan
28
29
30
31
32

33 All correspondence should get to Kazuo Ogawa, MD

34 Division of Cardiology, Department of Internal Medicine

35 The Jikei University School of Medicine

36 3-25-8, Nishi-Shinbashi, Minato-ku

37 Tokyo, Japan 105-8461

38 Telephone: +81-3-3433-1111

39 FAX: +81-3-3433-3459

40 E-mail: oga-n@jikei.ac.jp

41 This author takes responsibility for all aspects of the reliability and freedom from bias of the
42 data presented and their discussed interpretation
43
44
45
46
47
48
49

50 **Keywords:** MDA-LDL; Smoking; Oxidative stress; Coronary artery disease; Statin therapy
51

52
53 **Word count:** 2769
54
55
56
57
58
59
60

Abstract

Objectives:

MDA-LDL level is a marker of oxidative stress and is linked to progression of arteriosclerosis; however, the clinical factors affecting to the oxidized LDL level have not been elucidated. We herein investigated various factors to identify correlation with MDA-LDL level in high risk patients requiring catheter intervention.

Setting:

Secondary care (cardiology), single center study

Participants:

600 patients who were admitted to our hospital and underwent cardiac catheterization

Primary and secondary outcome measures:

Blood samples were obtained to measure lipid profiles and MDA-LDL level.

Results:

With regard to smoking status, MDA-LDL level was significantly higher in ex-/current smokers compared with non-smokers. Of note, there was no improvement of MDA-LDL level even in patients who quitted smoking. Multiple regression analysis showed that MDA-LDL level was positively correlated with LDL-cholesterol level, Brinkman index and male gender. The correlation between smoking status and either MDA-LDL or LDL-C level was investigated in

1
2
3
4
5
6 two groups; namely, patients, with or without statin treatment. In non-statin group, MDA-LDL
7
8
9 level and MDA-LDL/LDL-C ratio were significantly higher in ex-/current smokers compared
10
11
12 with non-smoker, while no significant correlation was observed between smoking status and
13
14
15 LDL-C level. In contrast, in statin group, there were no significant correlations between
16
17
18 smoking status and all any of these cholesterol parameters.
19

20
21 Conclusions:

22
23 We found that MDA-LDL level was affected by multiple factors, such as smoking status, LDL-
24
25
26 C level and male gender. The present findings give additional evidence that smoking should be
27
28
29 prohibited from MDA-LDL standpoint. Furthermore, statin therapy might have a beneficial
30
31
32 effect on the reduction of MDA-LDL level.
33

34
35 Trial registration:

36
37
38 N/A
39

40 41 42 43 **Main strengths**

44
45 Although oxidative LDL is associated with the marker of oxidative stress and the progression of
46
47
48 atherosclerosis, clinical factor which affects the oxidative LDL remains uncertain. Our study
49
50
51 revealed that MDA-LDL was associated with smoking and the MDA-LDL level would never
52
53
54 decrease with smoking cessation. However, MDA-LDL level was decreasing even in smokers
55
56
57 with statin therapy.
58

Study limitations

Smoking cessation was not found to be effective for reducing MDA-LDL level in this study; however, favorable effects of smoking cessation would likely occur with regard to other parameters than MDA-LDL level. Thus smoking cessation is recommended at any time, even after long-term smoking, and is considered to provide cardiovascular health benefits.

This was a retrospective study, and the true effects of a statin on MDA-LDL level remain unclear. Finally, we did not examine the prognosis of the study population and therefore, the effects of smoking cessation and/or statin therapy remains unclear especially in terms of their impact on MDA-LDL level. Prospective studies are required to obtain answers regarding these topics.

Introduction

The malondialdehyde modified low-density lipoprotein (MDA-LDL; oxidized LDL) is LDL that has been modified by MDA, leading to the production of a large amount of aldehyde when LDL becomes degenerated and oxidized¹.

It is known that MDA-LDL level is elevated in patients with dyslipidemia and diabetes mellitus (DM), both risks factors for atherosclerotic disease^{2,3}. Since MDA-LDL level has a positive correlation with the serum LDL level, the ratio of MDA-LDL/LDL-C (M/L) is used to evaluate the severity of oxidization of LDL; in some reports, not only MDA-LDL level, but also M/L ratio has been shown to increase in patients with DM compared with controls^{4,5}.

In patients with coronary artery disease (CAD), MDA-LDL level and M/L ratio have been shown to increase even when there are no other differences in the other lipid profiles⁶. In addition, it has been shown that the measurement of MDA-LDL level might be useful as a predictor of restenosis after percutaneous coronary intervention in patients with DM⁷. Based on these findings, it has been speculated that MDA-LDL level might be important marker of the progression of arteriosclerosis; however, the clinical factors possibly affecting MDA-LDL level have not been elucidated. Therefore, in the present study, we investigated “the clinical factors” affecting MDA-LDL level in high risk patients requiring catheter intervention.

Methods

Study patients

The study protocol (24-150[6916]) was approved by the ethics committee of the Jikei University School of Medicine. Six hundred consecutive patients who underwent cardiac catheterization from March 2010 to September 2011 were examined in this study. The baseline patient characteristics, including the clinical parameters and the biochemical data, were collected retrospectively from the hospital medical records. In addition, the results of the catheterization (i.e. the number of occluded or narrowed vessels), body weight, body mass index (BMI), coronary risk factors and medication profiles were also investigated. The patients taking eicosapentaenoic acid (EPA) were excluded since it has been demonstrated that EPA is major antihyperlipidemic agents with potent antioxidant effects.

Data collection

Blood sampling was performed to examine the serum MDA-LDL, serum creatinine, hemoglobin A1c (HbA1c), B-type (brain) natriuretic peptide (BNP) and low-density lipoprotein cholesterol (LDL-C) levels. MDA-LDL level was measured by an ELISA using an anti-MDA-LDL monoclonal antibody (ML25) and β -galactosidase anti-apoB monoclonal antibody (AB16)

¹. It is well known that the combination of ML25 and AB16 can accurately detect MDA-LDL ¹.

1
2
3
4
5
6 The concentration of MDA-LDL is defined at 1 mg/L of MDA-LDL produced artificially, which
7
8
9 shows the same signal as 1U/L of MDA-LDL in the serum. Diabetes mellitus (DM),
10
11
12 hypertension, dyslipidemia and smoking were defined as described previously^{8,9}. The definition
13
14
15 of smoking status was as follows: current smokers were those who were smoking at the time of
16
17
18 the study or who had smoked in the past year; the subjects who had quit smoking more than one
19
20
21 year before the study were defined as ex-smokers and those who had never smoked were
22
23
24 defined as non-smokers. Brinkman Index was used to evaluate the smoking status of current/ex-
25
26
27 smokers¹⁰.
28
29
30
31

32 *Statistical analysis*

33
34
35 Comparisons between MDA-LDL level and LDL-C level, age, BMI, HbA1c, Cr, BNP and
36
37
38 Brinkman index were performed with a linear regression analysis. Comparison between
39
40
41 Brinkman index and LDL-C was also performed with a linear regression analysis. Comparisons
42
43
44 of MDA-LDL levels between males and females, subjects with or without hypertension, non-
45
46
47 smokers versus ex-smokers versus current smokers, and among the various patient groups after
48
49
50 smoking cessation were performed with Mann-Whitney U test. Multiple factors, which were
51
52
53 considered to possibly modify MDA-LDL level were evaluated with a stepwise multiple
54
55
56 regression analysis. Comparisons of LDL-C and MDA-LDL level and M/L ratio between non-
57
58
59
60

1
2
3
4
5
6 smokers and smokers were performed with Mann-Whitney U test.
7
8

9 Statistical significance was defined as a value of $p < 0.05$.
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

Results

Baseline characteristics

The baseline characteristics of the patients in this study are shown in Table 1. The average age was 64.8 ± 11.4 years old and 80.3% of them were male. The percentages of non-smokers, ex-smokers and current smokers were 32%, 42% and 26%, respectively. The average LDL-C level and MDA-LDL level was 106.1 ± 30.8 mg/dl and 119.2 ± 48.7 U/L, respectively. The percentage of patients taking statin therapy was 55.3%.

Table 1-1 Patient's Characteristics

n=600	mean + SD
Age, years	64.8±11.4
Male, gender (%)	80.3
Height, cm	165.4±25.1
Weight, kg	66.8±13.3
BMI, kg/m ²	24.4±3.74
Non-smoker,n(%)	192(32.4)
Ex-smoker,n(%)	247(41.7)
Current smoker,n(%)	153(25.8)
Cr, mg/dL	1.38±1.94
eGFR, mL/min/1.73m ²	62.6±21.6
HbA1c, %	6.4±1.1
BNP, pg/mL	140±263
LDL-C,mg/dl	106.1±30.8
MDA-LDL,U/L	119.2±48.7
M/L	1.16±0.47

BMI: body mass index Cr: creatinine, eGFR: estimated glomerular filtration rate, BNP: B-type natriuretic peptide, LDL-C:low-density lipoprotein cholesterol ,MDA-LDL: Malondialdehyde modified low density lipoprotein. M/L: Malondialdehyde modified low density lipoprotein/ low density lipoprotein cholesterol,

Table 1-2 Patient's Characteristics

Disease	n(%)
Diabetes Mellitus	252(42.0)
Hypertension	455(75.5)

Dyslipidemia	441(73.5)
Medicine	n(%)
statin	332(55.3)
Ezetimibe	31(5.2)
fibrate	13(2.1)
Coronary Artery Disease	n(%)
0VD	215(35.8)
1VD	249(41.5)
2VD	84(14.0)
3VD	52(8.7)

0VD: 0-vessel disease, 1VD: single-vessel disease, 2VD: double-vessel disease, 3VD: triple-vessel disease

Clinical factors affecting MDA-LDL level

To elucidate the determinants of MDA-LDL level, we first performed a simple regression analysis (*Fig. 1*). MDA-LDL level showed a significantly positive correlation with LDL-C level (*Fig. 1A*) and a negative correlation with age (*Fig. 1B*). In addition, MDA-LDL levels were significantly higher in males as well as the patients without hypertension (*Figs. 1C, D*). BMI, HbA1c, Cr and BNP level had no impact on MDA-LDL level (*Figs. 1E-H*).

Correlation of smoking status with MDA-LDL level

We next examined the impact of smoking status on MDA-LDL level. MDA-LDL, but not LDL-C, showed a significantly positive correlation with the smoking profiles indicated by Brinkman

1
2
3
4
5
6 index (*Fig. 2-A, B*). The patients were subsequently divided into three groups according to their
7
8
9 smoking status: Non-, ex- and current smokers. MDA-LDL level was significantly higher in
10
11
12 both ex- and current smokers compared to that in non-smokers (*Fig. 2-C*). Moreover, when
13
14
15 patients were compared based on the number of years after smoking cessation, not only the
16
17
18 current smoking group, but also the group that had quit smoking within 10 years and that where
19
20
21 the patients had quit smoking more than 21 years earlier showed higher MDA-LDL levels than
22
23
24 did the non-smoking group (*Fig. 2-D*). This suggests that MDA-LDL level will never
25
26
27 completely recover once a subject has started smoking.
28
29
30
31

32 *Clinical factors affecting MDA-LDL level identified in multiple regression analysis*

33
34
35 To assess the independent determinants of MDA-LDL level, a multiple regression analysis was
36
37
38 performed. After removing the confounding factors, MDA-LDL level was shown to be
39
40
41 positively correlated with LDL-C level ($p < 0.001$), Brinkman index ($p = 0.009$) and a male gender
42
43
44 ($p = 0.019$) (Table 2).
45

46 **Table 2 Multiple Regression Analysis**
47

48	49	50	51	52	53	54
Significant variable	Regression coefficients	Standard error	Standard regression coefficients	F	p	
55	56	57	58	59	60	
LDL-C	0.675	0.06	0.429	128.089	<0.001	

Brinkman index	0.008	0.003	0.105	71.502	0.009
Gender	11.511	4.908	0.94	49.901	0.019

Objective variable: MDA-LDL

Explanatory variable: BMI, Age, Gender, brinkman index, Cr, BNP, LDL-C, HbA1c, HT

No significant variables: BMI, Age, Cr, BNP, HbA1c, HT

Effects of statin therapy on the correlations of smoking status with MDA-LDL or LDL-cholesterol level

The correlations of the smoking status with MDA-LDL/LDL-C level were investigated after patients were divided into two groups; those with or without statin treatment (*Fig. 3*). In non-statin-treated group, M-LDL level as well as MDA-LDL/LDL-C ratio, was significantly increased in ex-/current smokers compared to those in non-smokers, although there was no significant differences in LDL-C levels between the subjects with the different smoking status. LDL-C level was not significantly different in non-statin-treated and statin-treated groups.

Discussion

In this study, we investigated the factors associated with MDA-LDL level in high risk patients requiring cardiac catheterization. According to a multivariate analysis, Brinkman index, as well as the LDL-C level and gender were found to be significantly associated with MDA-LDL level. Furthermore, we found that smoking cessation was not effective for reducing MDA-LDL level,

1
2
3
4
5
6 even after the patients had quit smoking for many years. However, we found evidence that
7
8
9 statin treatment may reduce MDA-LDL level, which could possibly help in the treatment of
10
11
12 smokers.

13
14
15 It has been reported that smoking may affect susceptibility of plasma LDL to peroxidative
16
17
18 modification. Modified LDL has been shown to be the preferred substrate for macrophages and
19
20
21 induces their subsequent transformation into foam cells ^{11 12}. Thus, oxidative stress is very
22
23
24 important for synthesis of modified-LDL. However, it is noteworthy that MDA-LDL level was
25
26
27 emphatically influenced by smoking but not by obesity (estimated by BMI), hypertension,
28
29
30 diabetes mellitus (estimated by HbA1c level), renal failure (estimated by Cr level) or heart
31
32
33 failure (estimated by BNP level) by the multivariate analysis in this study, though all of these
34
35
36 clinical characteristics have been shown to increase oxidative stress. The precise mechanisms by
37
38
39 which smoking increases MDA-LDL level remain unclear at present.

40
41
42 The current study clearly showed that smoking was substantially harmful with regard to
43
44
45 increasing MDA-LDL level. When we examined the effect of smoking status on MDA-LDL
46
47
48 level among the non-smokers, ex-smokers and current smokers, we found that MDA-LDL level
49
50
51 was still higher in the ex-smokers than in the non-smokers and was unexpectedly similar to the
52
53
54 level in the current smokers. Furthermore, we examined the effects of the period of smoking
55
56
57 cessation and the analysis indicated that even many years after smoking cessation, there was no
58
59
60

1
2
3
4
5
6 significant reduction of MDA-LDL level. This result suggests that smoking should never be
7
8
9 started, and that if started, it is important to quit smoking as soon as possible before becoming a
10
11
12 heavy smokers.

13
14 The present results suggests that smoking keeps MDA-LDL level elevated for a long time.
15
16
17 Therefore, a method for lowering MDA-LDL level is especially needed for smokers. One
18
19
20 possible candidate would be statin therapy. Statins facilitate the LDL uptake in hepatocytes,
21
22
23 decrease old LDL (which is easily oxidized), and thereby reduce the risk of LDL oxidization ¹³.
24
25
26 The decreases in fatty acids and cholesterol in the lipoprotein are also likely to lead to a
27
28
29 decrease in oxidization ¹⁴. In the current study, we examined the effects of statin treatment on
30
31
32 MDA-LDL levels between non-smokers and smokers. MDA-LDL level was found to be
33
34
35 significantly higher in smokers than in non-smokers in the stain (-) group. On the other hand,
36
37
38 the effect of smoking on LDL-C level was not seen in the statin (+) group, the levels were
39
40
41 similar between smokers and non-smokers. The ratio of MDA-LDL/LDL-C showed a similar
42
43
44 result. These results may suggest that statin therapy can reduces MDA-LDL level in smokers to
45
46
47 a level similar to that in non-smokers.

48
49 It has been reported that cigarette smoking is one of the risk factors for organic stenosis but that
50
51
52 it does not act alone in contributing to the progression of atherosclerosis ^{14 15}. In addition
53
54
55 atherosclerosis was not produced by smoking alone in animal models ¹⁶. However, cigarette
56
57
58
59
60

1
2
3
4
5
6 smoking acts in concert with other risk factors such as hypercholesterolemia to accelerate
7
8 atherosclerosis^{14 15 17 18 19}. The current study is in agreement with the previous reports.
9
10
11 Importantly, it has been shown that among other risk factors cigarette smoking alone is a highly
12
13 significant risk factor for coronary spasm^{20 21 22 23 24} and treatment with fluvastatin reduced the
14
15 coronary spasm²⁵. Thus, smoking cessation combined with statin therapy would be beneficial
16
17 for prevention of ischemic heart disease by reducing progression of atherosclerosis and
18
19 suppressing coronary spasm.
20
21
22
23
24
25
26
27
28

29 **Conclusion**

30
31
32 We found that MDA-LDL level was affected by multiple factors such as smoking status (as
33
34 indicated by Brinkman index), LDL-C level and gender. In addition to its other health effects,
35
36 smoking should be strongly prohibited due to its harmful effects from MDA-LDL standpoint.
37
38

39
40 We recommend that patients should never smoke, but that once smoking has started, it is
41
42 essential to quit smoking as early as possible and to cut back on the number of cigarettes
43
44 consumed. Furthermore, statin therapy might have a beneficial effect on the reduction of MDA-
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
LDL level.

52 **Contributorship Statement:**

55
56
57
58
59
60
KO collected the data, performed the statistical analyses, and wrote the manuscript.

1
2
3
4
5
6 TT and TN conceived of the research hypothesis and analyses, wrote and edited the
7
8
9 manuscript. HS and SA performed the statistical analyses and edited the manuscript.
10
11
12 KM and TO participated in the design and coordination of the study and collected the
13
14
15 data. MY conceived of the study, and participated in its coordination and edited the
16
17
18 manuscript. All authors read and approved the final manuscript.
19

20
21 **Competing Interests:** None
22

23
24 **Data Sharing Statement:** No additional data are available
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

1. Kotani K, Maekawa M, Kanno T, et al. Distribution of immunoreactive malondialdehyde-modified low-density lipoprotein in human serum. *Biochimica et biophysica acta* 1994;**1215**(1-2):121-5.
2. Kitano S, Kanno T, Maekawa M, et al. Improved method for the immunological detection of malondialdehyde-modified low-density lipoproteins in human serum. *Analytica Chimica Acta* 2004;**509**(2):229-35.
3. Kondo A, Manabe M, Saito K, et al. Insulin treatment prevents LDL from accelerated oxidation in patients with diabetes. *Journal of atherosclerosis and thrombosis* 2002;**9**(6):280-7.
4. Kondo A, Muranaka Y, Ohta I, et al. Relationship between triglyceride concentrations and LDL size evaluated by malondialdehyde-modified LDL. *Clinical chemistry* 2001;**47**(5):893-900.
5. Kondo A, Li J, Manabe M, et al. Relationship between high-density lipoprotein-cholesterol and malondialdehyde-modified low-density lipoprotein concentrations. *Journal of atherosclerosis and thrombosis* 2003;**10**(2):72-8.
6. Tanaga K, Bujo H, Inoue M, et al. Increased circulating malondialdehyde-modified LDL levels in patients with coronary artery diseases and their association with peak sizes of LDL particles. *Arteriosclerosis, thrombosis, and vascular biology* 2002;**22**(4):662-6.
7. Shigematsu S, Takahashi N, Hara M, et al. Increased incidence of coronary in-stent restenosis in type 2 diabetic patients is related to elevated serum malondialdehyde-modified low-density lipoprotein. *Circulation journal : official journal of the Japanese Circulation Society* 2007;**71**(11):1697-702.
8. Yagi H, Komukai K, Hashimoto K, et al. Difference in risk factors between acute coronary syndrome and stable angina pectoris in the Japanese: smoking as a crucial risk factor of acute coronary syndrome. *Journal of cardiology* 2010;**55**(3):345-53.
9. Sekiyama H, Nagoshi T, Komukai K, et al. Transient decrease in serum potassium level during ischemic attack of acute coronary syndrome: paradoxical contribution of plasma glucose level and glycohemoglobin. *Cardiovascular diabetology* 2013;**12**:4.
10. Brinkman GL, Coates EO, Jr. The effect of bronchitis, smoking, and occupation on ventilation. *The American review of respiratory disease* 1963;**87**:684-93.
11. Yokode M, Kita T, Arai H, et al. Cholesteryl ester accumulation in macrophages incubated with low density lipoprotein pretreated with cigarette smoke extract. *Proceedings of the National Academy of Sciences of the United States of America*

- 1
2
3
4
5 1988;**85**(7):2344-8.
6
7 12. Harats D, Ben-Naim M, Dabach Y, et al. Cigarette smoking renders LDL susceptible to
8 peroxidative modification and enhanced metabolism by macrophages.
9 Atherosclerosis 1989;**79**(2-3):245-52.
10
11 13. Gotto AM. Interactions of the major risk factors for coronary heart disease. The
12 American Journal of Medicine 1986;**80**(2):48-55.
13
14 14. Willett WC, Green A, Stampfer MJ, et al. Relative and absolute excess risks of coronary
15 heart disease among women who smoke cigarettes. The New England journal of
16 medicine 1987;**317**(21):1303-9.
17
18 15. Stein Y, Harats D, Stein O. Why is smoking a major risk factor for coronary heart
19 disease in hyperlipidemic subjects? Annals of the New York Academy of Sciences
20 1993;**686**:66-9; discussion 69-71.
21
22 16. Asano M, Ohkubo C, Hirokawa A, et al. Smoking Research Foundation Annual Research
23 Report, On macro- and microcirculatory effects of tobacco smoke inhalation on
24 atherogenesis in the rabbit. 1987:251-61.
25
26 17. Anderson KM, Wilson PW, Odell PM, et al. An updated coronary risk profile. A
27 statement for health professionals. Circulation 1991;**83**(1):356-62.
28
29 18. Gotto AM, Jr. Interactions of the major risk factors for coronary heart disease. Am J
30 Med 1986;**80**(2a):48-55.
31
32 19. Holbrook JH, Grundy SM, Hennekens CH, et al. Cigarette smoking and cardiovascular
33 diseases. A statement for health professionals by a task force appointed by the
34 steering committee of the American Heart Association. Circulation
35 1984;**70**(6):1114a-17a.
36
37 20. Caralis DG, Deligonul U, Kern MJ, et al. Smoking is a risk factor for coronary spasm in
38 young women. Circulation 1992;**85**(3):905-9.
39
40 21. Sugiishi M, Takatsu F. Cigarette smoking is a major risk factor for coronary spasm.
41 Circulation 1993;**87**(1):76-9.
42
43 22. Yoshimura M, Yasue H, Nakayama M, et al. A missense Glu298Asp variant in the
44 endothelial nitric oxide synthase gene is associated with coronary spasm in the
45 Japanese. Human genetics 1998;**103**(1):65-9.
46
47 23. Nakayama M, Yasue H, Yoshimura M, et al. T-786->C mutation in the 5'-flanking
48 region of the endothelial nitric oxide synthase gene is associated with coronary
49 spasm. Circulation 1999;**99**(22):2864-70.
50
51 24. Takaoka K, Yoshimura M, Ogawa H, et al. Comparison of the risk factors for coronary
52 artery spasm with those for organic stenosis in a Japanese population: role of
53 cigarette smoking. International journal of cardiology 2000;**72**(2):121-6.
54
55
56
57
58
59
60

- 1
2
3
4
5 25. Yasue H, Mizuno Y, Harada E, et al. Effects of a 3-hydroxy-3-methylglutaryl coenzyme A
6 reductase inhibitor, fluvastatin, on coronary spasm after withdrawal of calcium-
7 channel blockers. *Journal of the American College of Cardiology* 2008;**51**(18):1742-8.
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

Figure legends

Fig. 1 Correlations of MDA-LDL level with various clinical factors

The clinical factors affecting MDA-LDL level are shown in (A)-(H).

Fig. 2 Correlation of smoking status with MDA-LDL and LDL-cholesterol levels

Correlation between MDA-LDL level and Brinkman index (A) and, between LDL-C level and Brinkman index (B) was determined with a linear regression analysis. Comparison of MDA-LDL levels among non-smokers, ex-smokers and current smokers (C). Comparison of MDA-LDL levels in each patient group among non-smokers and ex-smokers who had quit more than 21 years earlier, ex-smokers who had quit 11 to 20 years ago, ex-smokers who had quit less than 10 years ago and current smokers (D).

Fig. 3 Effects of the statin therapy on the correlations of smoking status with MDA-LDL or LDL-cholesterol level

The correlations of the smoking status with MDA-LDL and LDL-C levels were investigated after dividing the patients into two groups; with (A) or without (B) statin treatment.

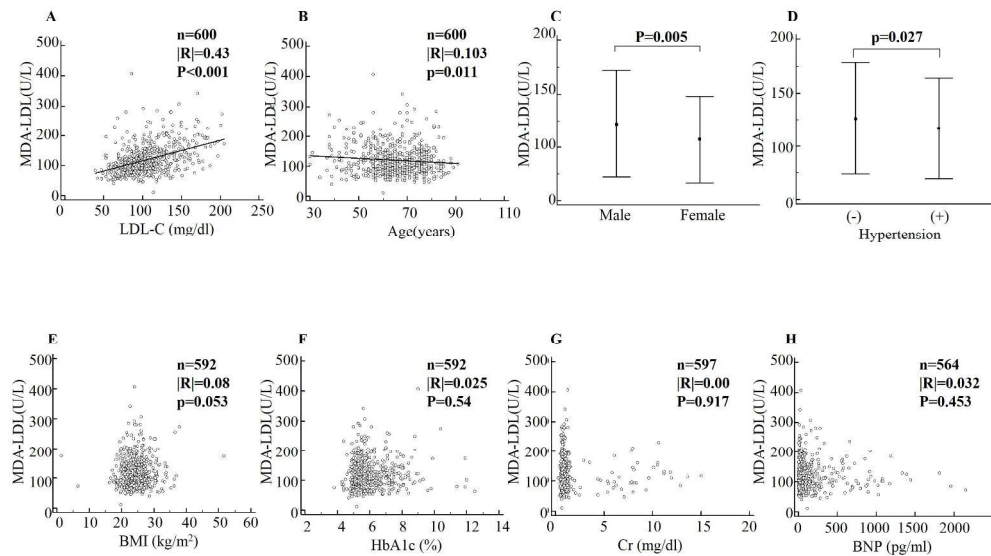


Figure 1

Fig. 1 Correlations of MDA-LDL level with various clinical factors /The clinical factors affecting MDA-LDL level are shown in (A)-(H).

254x190mm (300 x 300 DPI)

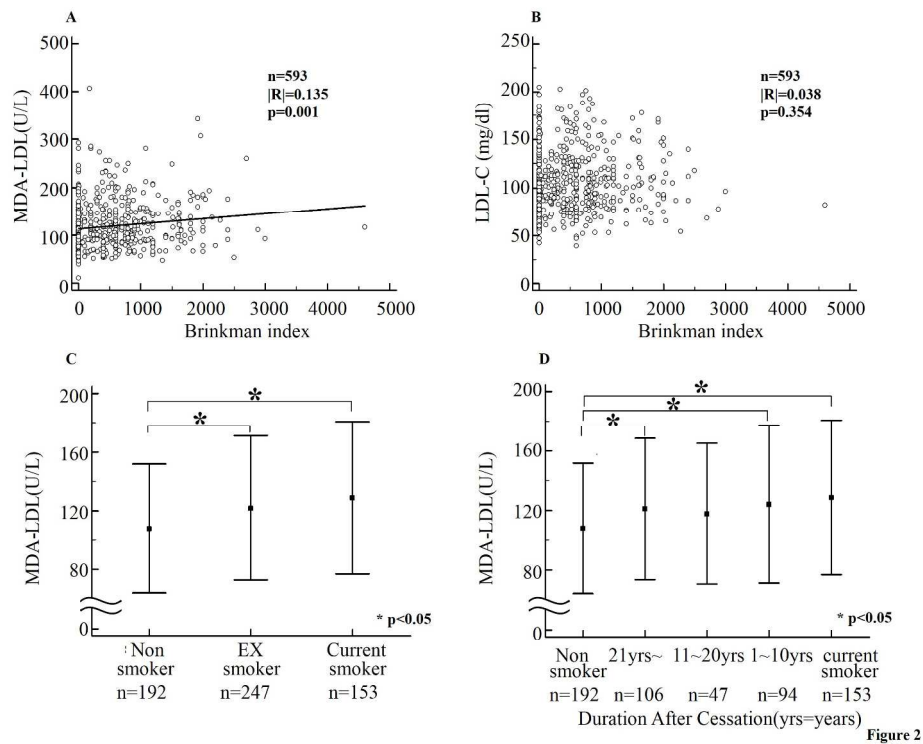


Figure 2

Fig. 2 Correlation of smoking status with MDA-LDL and LDL-cholesterol levels / Correlation between MDA-LDL level and Brinkman index (A) and, between LDL-C level and Brinkman index (B) was determined with a linear regression analysis. Comparison of MDA-LDL levels among non-smokers, ex-smokers and current smokers (C). Comparison of MDA-LDL levels in each patient group among non-smokers and ex-smokers who had quit more than 21 years earlier, ex-smokers who had quit 11 to 20 years ago, ex-smokers who had quit less than 10 years ago and current smokers (D).

254x190mm (300 x 300 DPI)

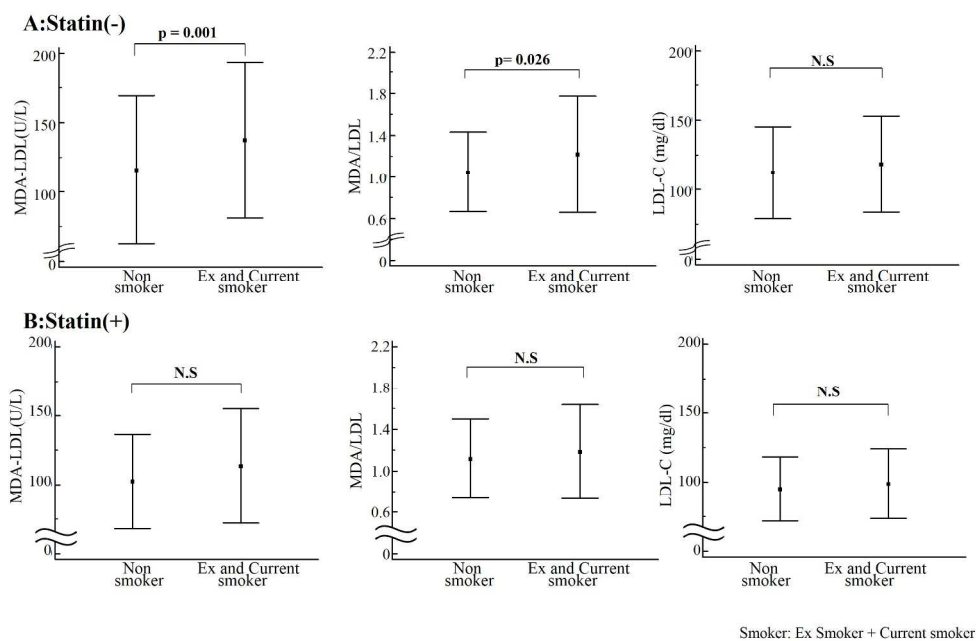


Figure 3

Fig. 3 Effects of the statin therapy on the correlations of smoking status with MDA-LDL or LDL-cholesterol level / The correlations of the smoking status with MDA-LDL and LDL-C levels were investigated after dividing the patients into two groups; with (A) or without (B) statin treatment.

254x190mm (300 x 300 DPI)

BMJ Open

Increase in the Oxidized Low-Density Lipoprotein Level by Smoking and the Possible Inhibitory Effect of Statin Therapy in Patients with Cardiovascular Disease.

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-005455.R1
Article Type:	Research
Date Submitted by the Author:	16-Nov-2014
Complete List of Authors:	Ogawa, Kazuo; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Tanaka, Toshikazu; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Nagoshi, Tomohisa; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Sekiyama, Hiroshi; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Arase, Satoshi; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Minai, Kosuke; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Ogawa, Takayuki; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine Yoshimura, Michihiro; The Jikei University School of Medicine, Division of Cardiology, Department of Internal Medicine
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Smoking and tobacco, Epidemiology
Keywords:	Coronary heart disease < CARDIOLOGY, Lipid disorders < DIABETES & ENDOCRINOLOGY, Cardiology < INTERNAL MEDICINE

SCHOLARONE™
Manuscripts

1
2
3
4
5
6 **Increase in the Oxidized Low-Density Lipoprotein Level by Smoking and the Possible**

7
8
9 **Inhibitory Effect of Statin Therapy in Patients with Cardiovascular Disease:**

10
11
12 **a Retrospective Study**

13
14
15
16
17
18 Kazuo Ogawa, MD. ; Toshikazu Tanaka, MD. PhD. ; Tomohisa Nagoshi, MD. PhD. ;

19
20
21 Hiroshi Sekiyama, MD. PhD. ; Satoshi Arase, MD. ; Kosuke Minai, MD. PhD. ;

22
23
24 Takayuki Ogawa, MD. PhD. and Michihiro Yoshimura, MD. PhD.

25
26
27 Division of Cardiology, Department of Internal Medicine,

28
29
30 The Jikei University School of Medicine, Tokyo, Japan

31
32
33
34
35 All correspondence should get to Kazuo Ogawa, MD

36
37 Division of Cardiology, Department of Internal Medicine

38
39 The Jikei University School of Medicine

40
41 3-25-8, Nishi-Shinbashi, Minato-ku

42
43 Tokyo, Japan 105-8461

44
45 Telephone: +81-3-3433-1111

46
47 FAX: +81-3-3433-3459

48
49 E-mail: oga-n@jikei.ac.jp

50
51
52 This author takes responsibility for all aspects of the reliability and freedom from bias of the
53 data presented and their discussed interpretation

54
55
56 **Keywords:** MDA-LDL; Smoking; Oxidative stress; Coronary artery disease; Statin therapy

57
58
59 **Word count:** 2650

Abstract

Objectives:

MDA-LDL level is a marker of oxidative stress and is linked to progression of arteriosclerosis; however, the clinical factors affecting to the oxidized LDL level have not been elucidated. We herein investigated various factors to identify correlation with MDA-LDL level in high risk patients requiring catheter intervention.

Setting:

Secondary care (cardiology), single center study

Participants:

600 patients who were admitted to our hospital and underwent cardiac catheterization

Primary and secondary outcome measures:

Blood samples were obtained to measure lipid profiles and MDA-LDL level.

Results:

With regard to smoking status, MDA-LDL level was significantly higher in ex-/current smokers compared with non-smokers. Of note, there was no improvement of MDA-LDL level even in patients who quitted smoking. Multiple regression analysis showed that MDA-LDL level was positively correlated with LDL-cholesterol level, Brinkman index and male gender. The correlation between smoking status and either MDA-LDL or LDL-C level was investigated in

1
2
3
4
5
6 two groups; namely, patients, with or without statin treatment. In non-statin group, MDA-LDL
7
8
9 level and MDA-LDL/LDL-C ratio were significantly higher in ex-/current smokers compared
10
11
12 with non-smoker, while no significant correlation was observed between smoking status and
13
14
15 LDL-C level. In contrast, in statin group, there were no significant correlations between
16
17
18 smoking status and all any of these cholesterol parameters.
19

20 Conclusions:

21
22
23 We found that MDA-LDL level was affected by multiple factors, such as smoking status,
24
25
26 LDL-C level and male gender. The present findings give additional evidence that smoking
27
28
29 should be prohibited from MDA-LDL standpoint. Furthermore, statin therapy might have a
30
31
32 beneficial effect on the reduction of MDA-LDL level.
33

34
35 Trial registration: N/A
36
37
38
39

40 **Main strengths**

41
42 Although oxidative LDL is associated with the marker of oxidative stress and the progression of
43
44
45 atherosclerosis, clinical factor which affects the oxidative LDL remains uncertain. Our study
46
47
48 revealed that MDA-LDL was associated with smoking and MDA-LDL level would never
49
50
51 decrease with smoking cessation. However, MDA-LDL level was decreasing even in smokers
52
53
54 with statin therapy.
55

56 **Study limitations**

1
2
3
4
5
6 Smoking cessation was not found to be effective for reducing MDA-LDL level in this study;
7
8
9 however, favorable effects of smoking cessation would likely occur with regard to other
10
11 parameters than MDA-LDL level. Thus smoking cessation is recommended at any time, even
12
13 after long-term smoking, and is considered to provide cardiovascular health benefits.
14
15

16
17 This was a retrospective study, and the true effect of statin on MDA-LDL level remains
18
19 uncertain. Finally, we did not examine the prognosis of the study population and therefore, the
20
21 effect of smoking cessation and/or statin therapy remains uncertain, especially in terms of their
22
23 impact on MDA-LDL level. Prospective studies should be required to obtain answers regarding
24
25 their topics.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

The malondialdehyde modified low-density lipoprotein (MDA-LDL; oxidized LDL) is LDL that has been modified by MDA, leading to the production of a large amount of aldehyde when LDL becomes degenerated and oxidized ¹.

It is known that MDA-LDL level is elevated in patients with dyslipidemia and diabetes mellitus (DM), both risks factors for atherosclerotic disease ^{2,3}. Since MDA-LDL level has a positive correlation with the serum LDL level, the ratio of MDA-LDL/LDL-C (M/L) is used to evaluate the severity of oxidization of LDL; in some reports, not only MDA-LDL level, but also M/L ratio has been shown to increase in patients with DM compared with controls ^{4,5}.

In patients with coronary artery disease (CAD), MDA-LDL level and M/L ratio have been shown to increase even when there are no other differences in the other lipid profiles ⁶. In addition, it has been shown that the measurement of MDA-LDL level might be useful as a predictor of restenosis after percutaneous coronary intervention in patients with DM ⁷. Based on these findings, it has been speculated that MDA-LDL level might be important marker of the progression of arteriosclerosis; however, the clinical factors possibly affecting MDA-LDL level have not been elucidated. Therefore, in the present study, we investigated “the clinical factors” affecting MDA-LDL level in high risk patients requiring catheter intervention.

Methods

Study patients

Six hundred consecutive patients who underwent cardiac catheterization from March 2010 to September 2011 were examined in this study. The baseline patient characteristics, including the clinical parameters and the biochemical data, were collected retrospectively from the hospital medical records. In addition, the results of the catheterization (i.e. the number of occluded or narrowed vessels), body weight, body mass index (BMI), coronary risk factors and medication profiles were also investigated. The patients taking eicosapentaenoic acid (EPA) were excluded since it has been demonstrated that EPA is major lipid-lowering agents with potent antioxidant effects. This study was approved by the ethics committee of the Jikei University School of Medicine (Study protocol: 24-150[6916]); and we complied with the routine ethical regulation of our institution as follows. This is a retrospective study and the informed consent could not be obtained from each patient. Instead of informed consent from each patient, we publicly posted a notice about the study design and contact information at a publicly-known space in our institution.

Data collection

Blood sampling was performed to examine the serum MDA-LDL, serum creatinine,

1
2
3
4
5
6 hemoglobin A1c (HbA1c), B-type (brain) natriuretic peptide (BNP) and low-density lipoprotein
7
8
9 cholesterol (LDL-C) levels. The previous study reported by Tismikas et al.⁸ encompassed that
10
11
12 PCI would affect the oxidative LDL level. To avoid the modification of LDL level by PCI, we
13
14
15 tried to take a blood draw immediately before the cardiac catheterization or at the outpatient
16
17
18 clinic before admission. MDA-LDL level was measured by an ELISA using an anti-MDA-LDL
19
20
21 monoclonal antibody (ML25) and β -galactosidase anti-apoB monoclonal antibody (AB16)¹. It
22
23
24 is well known that the combination of ML25 and AB16 can accurately detect MDA-LDL¹. The
25
26
27 concentration of MDA-LDL is defined at 1 mg/L of MDA-LDL produced artificially, which
28
29
30 shows the same signal as 1U/L of MDA-LDL in the serum. Serum levels of LDL cholesterol
31
32
33 was determined enzymatically. (Sekisui Medical Co., Ltd., Tokyo, Japan)

34
35 Diabetes mellitus (DM), hypertension and smoking were defined as described previously^{9 10}.
36
37
38 Dyslipidemia was diagnosed with the use of lipid-lowering agents, the presence of 1 or more
39
40
41 of the following 3 lipid disorders at first fasting blood sampling or both: a low-density
42
43
44 lipoprotein (LDL) cholesterol level ≥ 140 mg/dL, a triglyceride level ≥ 150 mg/dL, and a
45
46
47 high-density lipoprotein (HDL) cholesterol level < 40 mg/dL⁹. Blood sampling was performed
48
49
50 on the day of the catheter examination, except for the case that has already been performed at
51
52
53 outpatient clinic. Among 600 patients, 342 were performed on the day of the catheter
54
55
56 examination, and remaining 258 were at outpatient.
57
58
59
60

1
2
3
4
5
6 The definition of smoking status is as follows: current smokers were those who were smoking at
7
8
9 the time of the study or who had smoked in the past year; the subjects who had quit smoking
10
11
12 more than one year before the study were defined as ex-smokers and those who had never
13
14
15 smoked were defined as non-smokers. Brinkman Index was used to evaluate the smoking status
16
17
18 of current/ex-smokers¹¹.
19

20 21 22 23 *Statistical analysis*

24
25
26 Comparisons between MDA-LDL level and LDL-C level, age, BMI, HbA1c, Cr, BNP and
27
28
29 Brinkman index were performed with a linear regression analysis. Comparison between
30
31
32 Brinkman index and LDL-C was also performed with a linear regression analysis. Comparisons
33
34
35 of MDA-LDL level between males and females, subjects with or without hypertension,
36
37
38 non-smokers versus ex-smokers versus current smokers, and among the various patient groups
39
40
41 after smoking cessation were performed with Mann-Whitney U test. Kruskal Wallis test was
42
43
44 performed to evaluate the difference of MDA-LDL (C) and LDL-C (D) among 4 groups divided
45
46
47 by Brinkman index. Multiple factors, which were considered to possibly modify MDA-LDL
48
49
50 levels were evaluated with a stepwise multiple regression analysis. Comparison of LDL-C and
51
52
53 MDA-LDL level and M/L ratio between non-smokers and smokers were performed with
54
55
56 Mann-Whitney U test.
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

A value of $p < 0.05$ was considered to be statistically significant for all data that were statistically analyzed using the SPSS software package, version 21.0 (SPSS Inc., Chicago, IL).

For peer review only

Results

Baseline characteristics

The baseline characteristics of the patients in this study were shown in Table 1. The average age was 64.8 ± 11.4 years old and 80.3% of them were male. The percentages of non-smokers, ex-smokers and current smokers were 32%, 42% and 26%, respectively. The average LDL-C level and MDA-LDL level were 106.1 ± 30.8 mg/dl and 119.2 ± 48.7 U/L, respectively. The percentage of patients taking statin therapy was 55.3%. In addition, patient characteristics divided by smoking status into 3 groups (non-smokers, ex-smokers, current smokers) were shown in Table 1-3.

Table 1-1 Patient's Characteristics

	N=600	mean \pm SD
Age, years		64.8 \pm 11.4
Male, gender (%)		80.3
Height, cm		165.4 \pm 25.1
Weight, kg		66.8 \pm 13.3
BMI, kg/m ²		24.4 \pm 3.74
Non-smoker, N (%)		192(32.4)
Ex-smoker, N (%)		247(41.7)
Current smoker, N (%)		153(25.8)
Cr, mg/dL		1.38 \pm 1.94
eGFR, mL/min/1.73m ²		62.6 \pm 21.6
HbA1c, %		6.4 \pm 1.1
BNP, pg/mL		140 \pm 263
LDL-C, mg/dl		106.1 \pm 30.8
MDA-LDL,U/L		119.2 \pm 48.7
M/L		1.16 \pm 0.47

BMI: body mass index, Cr: creatinine, eGFR: estimated glomerular filtration rate, BNP: B-type natriuretic peptide, LDL-C: low-density lipoprotein cholesterol, MDA-LDL: malondialdehyde modified low density lipoprotein, M/L: malondialdehyde modified low density lipoprotein/ low density lipoprotein cholesterol

Table 1-2 Patient's characteristics

Disease	N (%)
Diabetes Mellitus	252(42.0)
Hypertension	455(75.5)
Dyslipidemia	441(73.5)
Medicine	N (%)
Statin	332(55.3)
Ezetimibe	31(5.2)
Fibrate	13(2.1)
Coronary Artery Disease	N (%)
0VD	215(35.8)
1VD	249(41.5)
2VD	84(14.0)
3VD	52(8.7)

0VD: 0-vessel disease, 1VD: single-vessel disease, 2VD: double-vessel disease, 3VD: triple-vessel disease

Table 1-3 Patient's characteristics divided by smoking status

	Non-smoker	Ex-smoker	Current smoker
Number of patients (%)	192(32.4)	250(42.2)	151(25.4)
Age	66.9±12.4	65.5±9.5	60.9±12.2
Male, gender (%)	110(57.3)	228(91.2) *	138(91.4)
Height, cm	160.3±11.0	166.0±11.8*	167.4±7.3
Weight, kg	62.7±14.0	67.7±10.9*	70.6±14.8
BMI, kg/m ²	24.2±3.6	24.3±3.0	25.0±4.9
Cr, mg/dL	1.2±1.5	1.6±2.1	1.3±2.1

HbA1c, %	6.3±0.9	6.4±1.1	6.4±1.1
BNP, pg/mL	167.1±302.0	133.2±264.8	120.7±201.1
LDL-C, mg/dL	102.3±29.1	104.2±28.4	114.8±35.2**
MDA-LDL, U/L	108.0±43.6	122.1±49.2*	129.3±52.1
M/L	2.06±0.85	2.26±0.82	2.6±0.99
LVEF, %	60.2±10.0	56.7±9.6*	54.4±12.1
Diabetes Mellitus	72(37.5)	112(44.8)	65(43.0) *
Hypertension	155(80.7)	191(76.4)	104(68.9) *
Dyslipidemia	134(69.8)	185(74.0)	116(76.8)
Statin	111(57.8)	146(58.4)	73(48.3)
Ezetimibe	10(3.6)	14(5.6)	7(4.6)
Fibrate	2(1.0)	6(2.4)	5(3.3)
Coronary Artery Disease			
0VD	82(42.7)	83(33.2)	49(32.5)
1VD	69(35.9)	105(42.0)	71(47.0) *
2VD	26(13.5)	34(42.0)	22(14.6)
3VD	15(7.8)	28(7.2)	9(6.0)

*: P<0.05 vs. Non-smoker, **: P<0.05 vs. Ex-smoker

Clinical factors affecting MDA-LDL level

To elucidate the determinants of MDA-LDL level, a simple regression analysis was performed.

(Fig. 1) MDA-LDL level showed a significantly positive correlation with LDL-C level (Fig.

1A) and a negative correlation with age. (Fig. 1B) In addition, MDA-LDL level was

1
2
3
4
5
6 significantly higher in male as well as patient without hypertension. (Figs. 1C, D) BMI, HbA1c,
7
8
9 Cr and BNP level had no impact on MDA-LDL level. (Figs. 1E-H)

10 11 12 13 14 15 *Correlation of smoking status with MDA-LDL level*

16
17
18 Next, the impact of smoking status on MDA-LDL level was performed. MDA-LDL, but not
19
20
21 LDL-C, showed a significantly positive correlation with the smoking profiles indicated by
22
23
24 Brinkman index. (Fig. 2-1-A, B) However, considering the bias of the Brinkman distribution, in
25
26
27 addition to Fig. 2-1-A, B, MDA-LDL level and LDL-C level were analyzed with dividing by
28
29
30 Brinkman index into 4 groups. There was a significant difference of MDA-LDL level between
31
32
33 non-smoker and current-smoker, whereas there was no significant difference of LDL-C
34
35
36 regardless of the smoking status. (Fig. 2-1C, D)

37
38 The patients were subsequently divided into three groups according to their smoking status:
39
40
41 Non-, ex- and current smokers. MDA-LDL level was significantly higher in both ex- and
42
43
44 current smokers compared to that in non-smokers. (Fig. 2-2-E) Moreover, when patients were
45
46
47 compared based on the number of years after smoking cessation, not only current smoking
48
49
50 group, but also group that had quit smoking within 10 years and that where the patients had quit
51
52
53 smoking more than 21 years earlier showed higher MDA-LDL levels than did non-smoking
54
55
56 group (Fig. 2-2-F).

Clinical factors affecting MDA-LDL level identified in multiple regression analysis

To assess the independent determinants of MDA-LDL level, a multiple regression analysis was performed. After removing the confounding factors, MDA-LDL level was shown to be positively correlated with LDL-C level ($p<0.001$), Brinkman index ($p=0.009$) and a male gender ($p=0.019$) (Table 2).

Table 2 Multiple Regression Analysis

Significant variable	Regression coefficients	Standard error	Standard regression coefficients	F	p
LDL-C	0.675	0.06	0.429	128.089	<0.001
Brinkman index	0.008	0.003	0.105	71.502	0.009
Gender	11.511	4.908	0.94	49.901	0.019

Objective variable: MDA-LDL

Explanatory variable: BMI, Age, Gender, brinkman index, Cr, BNP, LDL-C, HbA1c, HT

No significant variables: BMI, Age, Cr, BNP, HbA1c, HT

Effects of statin therapy on the correlation of smoking status with MDA-LDL or

LDL-cholesterol level

The correlations of smoking status with MDA-LDL/LDL-C level was investigated after patients were divided into two groups; those with or without statin treatment (*Fig.3*). In

1
2
3
4
5
6 non-statin-treated group, MDA-LDL level as well as MDA-LDL/LDL-C ratio, was significantly
7
8
9 increased in ex-/current smokers compared to those in non-smokers, although there was no
10
11
12 significant difference in LDL-C level between the subjects with the different smoking status.
13
14
15 LDL-C level was not significantly different in non-statin-treated and statin-treated groups.
16
17
18
19

20 21 **Discussion**

22
23 In this study, we investigated the factors associated with MDA-LDL level in high risk patients
24
25 requiring cardiac catheterization. According to a multivariate analysis, Brinkman index, as well
26
27 as the LDL-C level and gender were found to be significantly associated with MDA-LDL level.
28
29
30 Furthermore, we found that smoking cessation was not effective for reducing MDA-LDL level,
31
32
33 even after the patients had quit smoking for many years. However, we found evidence that statin
34
35
36 treatment may reduce MDA-LDL level, which could possibly help in the treatment of smokers.
37
38

39
40 It has been reported that smoking may affect susceptibility of plasma LDL to peroxidative
41
42 modification. Modified LDL has been shown to be the preferred substrate for macrophages and
43
44 induces their subsequent transformation into foam cells^{12 13}. Thus, oxidative stress is very
45
46
47 important for synthesis of modified-LDL. However, it is noteworthy that MDA-LDL level was
48
49
50 emphatically influenced by smoking but not by obesity (estimated by BMI), hypertension,
51
52
53 diabetes mellitus (estimated by HbA1c level), renal failure (estimated by Cr level) or heart
54
55
56
57
58
59
60

1
2
3
4
5
6 failure (estimated by BNP level) by the multivariate analysis in this study, though all of these
7
8
9 clinical characteristics have been shown to increase oxidative stress. The precise mechanism by
10
11
12 which smoking increases MDA-LDL level remains unclear at present.

13
14
15 The current study clearly showed that smoking was substantially harmful with regard to
16
17
18 increasing MDA-LDL level. When we examined the effect of smoking status on MDA-LDL
19
20
21 level among non-smokers, ex-smokers and current smokers, we found that MDA-LDL level was
22
23
24 still higher in ex-smokers than in non-smokers and was unexpectedly similar to the level in
25
26
27 current smokers. Furthermore, we examined the effects of the period of smoking cessation and
28
29
30 the analysis indicated that even many years after smoking cessation, there was no significant
31
32
33 reduction of MDA-LDL level. This result suggests that smoking should never be started, and
34
35
36 that if started, it is important to quit smoking as soon as possible before becoming a heavy
37
38
39 smoker.

40
41 The present result suggests that smoking keeps MDA-LDL level elevated for a long time.
42
43
44 Therefore, a method for lowering MDA-LDL level is especially needed for smokers. One
45
46
47 possible candidate might be statin therapy. Statins facilitate the LDL uptake in hepatocytes,
48
49
50 decrease old LDL (which is easily oxidized), and thereby reduce the risk of LDL oxidization ¹⁴.
51
52
53 The decreases in fatty acids and cholesterol in the lipoprotein are also likely to lead to a
54
55
56 decrease in oxidization ¹⁵. In the current study, we examined the effect of statin treatment on
57
58
59
60

1
2
3
4
5
6 MDA-LDL level between non-smokers and smokers. MDA-LDL level was found to be
7
8
9 significantly higher in smokers than in non-smokers in stain (-) group. On the other hand, the
10
11
12 effect of smoking on LDL-C level was not seen in statin (+) group, the level was similar
13
14
15 between smokers and non-smokers. The ratio of MDA-LDL/LDL-C showed a similar result.
16
17
18 These results may suggest that statin therapy would reduce MDA-LDL level in smokers to a
19
20
21 level similar to that in non-smokers.

22
23
24 It has been reported that cigarette smoking is one of the risk factors for organic stenosis but that
25
26
27 it does not act alone in contributing to the progression of atherosclerosis^{15 16}. In addition
28
29
30 atherosclerosis was not produced by smoking alone in animal models¹⁷. However, cigarette
31
32
33 smoking acts in concert with other risk factors such as hypercholesterolemia to accelerate
34
35
36 atherosclerosis^{15 16 18 19 20}. The current study is in agreement with the previous reports.
37
38
39 Importantly, it has been shown that among other risk factors cigarette smoking alone is a highly
40
41
42 significant risk factor for coronary spasm^{21 22 23 24 25} and treatment with fluvastatin reduced the
43
44
45 coronary spasm²⁶. Thus, smoking cessation combined with statin therapy would be beneficial
46
47
48 for prevention of ischemic heart disease by reducing progression of atherosclerosis and
49
50
51 suppressing coronary spasm.

52
53
54 In the present study, we performed multivariate analysis for determination of MDA-LDL by
55
56
57 using the factors of LDL-C level, age, BMI, HbA1c, Cr, BNP and Brinkman index; however,
58
59
60

1
2
3
4
5
6 there may be other factors associated with MDA-LDL in a direct or indirect manner. As a matter
7
8
9 of fact, the previous study by Matsuda et al. showed that MDA-LDL level was correlated with
10
11 triglyceride, HDL-C, metformin and α -glucosidase inhibitors in statin-treated diabetes patients
12
13 with CAD²⁷. Then, we performed another multivariate analysis. As a result, it revealed that
14
15 MDA-LDL level was correlated with LDL-C, triglyceride and smoking, however, not with
16
17 HDL-C, anti-hypertensive drugs, anti-diabetes agents such as metformin and α -glucosidase
18
19 inhibitors, number of the vessels with CAD and CRP. (Precise data not shown) Furthermore,
20
21 there may be confounding factors among them; and the correlation among each factors were
22
23 also investigated. As a result, there were only slight correlation between CAD and HDL-C
24
25 (R=-0.163), between CAD and smoking (R=0.098), between triglyceride and HDL-C (R=0.203)
26
27 and between triglyceride and smoking (R=-0.163) (Precise data not shown). The reason of the
28
29 difference between the previous study and ours may be due to the different study population. In
30
31 any case, it would be safe to say that smoking affected MDA-LDL level in a fairly direct
32
33 manner.
34
35
36
37
38
39
40
41
42
43
44
45

46 **Conclusion**

47
48
49 We found that MDA-LDL level was affected by multiple factors such as smoking status (as
50
51 indicated by Brinkman index), LDL-C level and gender. In addition to its other health effects,
52
53 smoking should be strongly prohibited due to its harmful effect from a MDA-LDL standpoint.
54
55
56
57
58
59
60

1
2
3
4
5
6 We recommend that patients should never smoke, but that once smoking has started, it is
7
8
9 essential to quit smoking as early as possible and to cut back on the number of cigarettes
10
11
12 consumed. Furthermore, statin therapy might have a beneficial effect on the reduction of
13
14
15 MDA-LDL level.
16
17
18
19

20 **Footnotes**

21
22
23 Contributors: Conceived and designed the experiments: KO TT KM MY. Performed the
24
25
26 experiments: KO HS SA TN TO. Performed the statistical analysis: KO HS KM MY.
27

28
29 Contributed reagents/materials/analysis tools: KO TT TN KM. Wrote the paper: KO TT KM
30
31
32 MY.
33
34
35
36
37

38 Competing interests: None
39
40
41
42

43 Funding: None
44
45
46
47
48

49 Ethics approval: The study protocol (24-150[6916]) was approved by the ethics committee of
50
51
52 the Jikei University School of Medicine.
53
54
55
56
57
58
59
60

Provenance and peer review: Not commissioned; externally peer reviewed

Data sharing: No additional data are available.

References

1. Kotani K, Maekawa M, Kanno T, et al. Distribution of immunoreactive malondialdehyde-modified low-density lipoprotein in human serum. *Biochimica et biophysica acta* 1994;**1215**(1-2):121-5.
2. Kitano S, Kanno T, Maekawa M, et al. Improved method for the immunological detection of malondialdehyde-modified low-density lipoproteins in human serum. *Analytica Chimica Acta* 2004;**509**(2):229-35.
3. Kondo A, Manabe M, Saito K, et al. Insulin treatment prevents LDL from accelerated oxidation in patients with diabetes. *Journal of atherosclerosis and thrombosis* 2002;**9**(6):280-7.
4. Kondo A, Muranaka Y, Ohta I, et al. Relationship between triglyceride concentrations and LDL size evaluated by malondialdehyde-modified LDL. *Clinical chemistry* 2001;**47**(5):893-900.
5. Kondo A, Li J, Manabe M, et al. Relationship between high-density lipoprotein-cholesterol and malondialdehyde-modified low-density lipoprotein concentrations. *Journal of atherosclerosis and thrombosis* 2003;**10**(2):72-8.
6. Tanaga K, Bujo H, Inoue M, et al. Increased circulating malondialdehyde-modified LDL levels in patients with coronary artery diseases and their association with peak sizes of LDL particles. *Arteriosclerosis, thrombosis, and vascular biology* 2002;**22**(4):662-6.
7. Shigematsu S, Takahashi N, Hara M, et al. Increased incidence of coronary in-stent restenosis in type 2 diabetic patients is related to elevated serum malondialdehyde-modified low-density lipoprotein. *Circulation journal : official journal of the Japanese Circulation Society* 2007;**71**(11):1697-702.
8. Tsimikas S, Lau HK, Han KR, et al. Percutaneous coronary intervention results in acute increases in oxidized phospholipids and lipoprotein(a): short-term and long-term immunologic responses to oxidized low-density lipoprotein. *Circulation* 2004;**109**(25):3164-70.
9. Yagi H, Komukai K, Hashimoto K, et al. Difference in risk factors between acute coronary syndrome and stable angina pectoris in the Japanese: smoking as a crucial risk factor of acute coronary syndrome. *Journal of cardiology* 2010;**55**(3):345-53.
10. Sekiyama H, Nagoshi T, Komukai K, et al. Transient decrease in serum potassium level during ischemic attack of acute coronary syndrome: paradoxical contribution of plasma glucose level

- and glycohemoglobin. *Cardiovascular diabetology* 2013;**12**:4.
11. Brinkman GL, Coates EO, Jr. The effect of bronchitis, smoking, and occupation on ventilation. *The American review of respiratory disease* 1963;87:684-93.
 12. Yokode M, Kita T, Arai H, et al. Cholesteryl ester accumulation in macrophages incubated with low density lipoprotein pretreated with cigarette smoke extract. *Proceedings of the National Academy of Sciences of the United States of America* 1988;85(7):2344-8.
 13. Harats D, Ben-Naim M, Dabach Y, et al. Cigarette smoking renders LDL susceptible to peroxidative modification and enhanced metabolism by macrophages. *Atherosclerosis* 1989;79(2-3):245-52.
 14. Gotto AM. Interactions of the major risk factors for coronary heart disease. *The American Journal of Medicine* 1986;80(2):48-55.
 15. Willett WC, Green A, Stampfer MJ, et al. Relative and absolute excess risks of coronary heart disease among women who smoke cigarettes. *The New England journal of medicine* 1987;317(21):1303-9.
 16. Stein Y, Harats D, Stein O. Why is smoking a major risk factor for coronary heart disease in hyperlipidemic subjects? *Annals of the New York Academy of Sciences* 1993;686:66-9; discussion 69-71.
 17. Asano M, Ohkubo C, Hirokawa A, et al. Smoking Research Foundation Annual Research Report, On macro- and microcirculatory effects of tobacco smoke inhalation on atherogenesis in the rabbit. 1987:251-61.
 18. Anderson KM, Wilson PW, Odell PM, et al. An updated coronary risk profile. A statement for health professionals. *Circulation* 1991;83(1):356-62.
 19. Gotto AM, Jr. Interactions of the major risk factors for coronary heart disease. *Am J Med* 1986;80(2a):48-55.
 20. Holbrook JH, Grundy SM, Hennekens CH, et al. Cigarette smoking and cardiovascular diseases. A statement for health professionals by a task force appointed by the steering committee of the American Heart Association. *Circulation* 1984;70(6):1114a-17a.
 21. Caralis DG, Deligonul U, Kern MJ, et al. Smoking is a risk factor for coronary spasm in young women. *Circulation* 1992;85(3):905-9.
 22. Sugiishi M, Takatsu F. Cigarette smoking is a major risk factor for coronary spasm. *Circulation* 1993;87(1):76-9.
 23. Yoshimura M, Yasue H, Nakayama M, et al. A missense Glu298Asp variant in the endothelial nitric oxide synthase gene is associated with coronary spasm in the Japanese. *Human genetics* 1998;103(1):65-9.
 24. Nakayama M, Yasue H, Yoshimura M, et al. T-786-->C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. *Circulation* 1999;99(22):2864-70.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
25. Takaoka K, Yoshimura M, Ogawa H, et al. Comparison of the risk factors for coronary artery spasm with those for organic stenosis in a Japanese population: role of cigarette smoking. *International journal of cardiology* 2000;72(2):121-6.
26. Yasue H, Mizuno Y, Harada E, et al. Effects of a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, fluvastatin, on coronary spasm after withdrawal of calcium-channel blockers. *Journal of the American College of Cardiology* 2008;51(18):1742-8.
27. Matsuda M, Tamura R, Kanno K, et al. Impact of dyslipidemic components of metabolic syndrome, adiponectin levels, and anti-diabetes medications on malondialdehyde-modified low-density lipoprotein levels in statin-treated diabetes patients with coronary artery disease. *Diabetology & metabolic syndrome* 2013;5(1):77.

Figure legends

Fig. 1 Correlations of MDA-LDL level with various clinical factors

The clinical factors affecting MDA-LDL level are shown in (A)-(H).

Fig. 2 Correlation of smoking status with MDA-LDL and LDL-cholesterol levels

Correlation between MDA-LDL level and Brinkman index (A) and, between LDL-C level and Brinkman index (B) was determined with a linear regression analysis. Kruskal Wallis test was performed to evaluate the difference of MDA-LDL (C) and LDL-C (D) among 4 groups divided by Brinkman index. Comparison of MDA-LDL levels among non-smokers, ex-smokers and current smokers (E). Comparison of MDA-LDL levels in each patient group among non-smokers and ex-smokers who had quit more than 21 years earlier, ex-smokers who had quit 11 to 20 years ago, ex-smokers who had quit less than 10 years ago and current smokers (F).

Fig. 3 Effects of the statin therapy on the correlations of smoking status with MDA-LDL or LDL-cholesterol level

The correlations of the smoking status with MDA-LDL and LDL-C levels were investigated after dividing the patients into two groups; with (A) or without (B) statin treatment.

1
2
3
4
5
6
7
8
9 **Increase in the Oxidized Low-Density Lipoprotein Level by Smoking and the Possible**

10
11 **Inhibitory Effect of Statin Therapy in Patients with Cardiovascular Disease.**

12
13
14
15
16 Kazuo Ogawa, MD. ; Toshikazu Tanaka, MD. PhD. ; Tomohisa Nagoshi, MD. PhD.;

17
18 Hiroshi Sekiyama, MD. PhD. ; Satoshi Arase, MD. ; Kosuke Minai, MD. PhD. ;

19
20
21 Takayuki Ogawa, MD. PhD and Michihiro Yoshimura, MD. PhD.

22
23
24 Division of Cardiology, Department of Internal Medicine,

25
26
27 The Jikei University School of Medicine, Tokyo, Japan

28
29
30
31
32 All correspondence should get to Kazuo Ogawa, MD
33 Division of Cardiology, Department of Internal Medicine
34 The Jikei University School of Medicine
35 3-25-8, Nishi-Shinbashi, Minato-ku
36 Tokyo, Japan 105-8461
37 Telephone: +81-3-3433-1111
38 FAX: +81-3-3433-3459
39 E-mail: oga-n@jikei.ac.jp

40
41
42 This author takes responsibility for all aspects of the reliability and freedom from bias of the
43 data presented and their discussed interpretation

44
45
46
47 | **Keywords:** MDA-LDL; **s**Smoking; **o**Oxidative stress; **c**Coronary artery disease; **s**Statin therapy

48
49
50 | **Word count:** [27692650](#)

Abstract

Objectives:

MDA-LDL level is a marker of oxidative stress and is linked to progression of arteriosclerosis; however, the clinical factors affecting the oxidized LDL level have not been elucidated. We herein investigated various factors to identify correlation with MDA-LDL level in high risk patients requiring catheter intervention.

Setting:

Secondary care (cardiology), single center study

Participants:

600 patients who were admitted to our hospital and underwent cardiac catheterization

Primary and secondary outcome measures:

Blood samples were obtained to measure lipid profiles and MDA-LDL level.

Results:

With regard to smoking status, MDA-LDL level was significantly higher in ex-/current smokers compared with non-smokers. Of note, there was no improvement of MDA-LDL level even in patients who quitted smoking. Multiple regression analysis showed that MDA-LDL level was positively correlated with LDL-cholesterol level, Brinkman index and male gender. The correlation between smoking status and either MDA-LDL or LDL-C level was investigated in

1
2
3
4
5
6
7
8
9 two groups; namely, patients, with or without statin treatment. In non-statin group, MDA-LDL
10
11 level and MDA-LDL/LDL-C ratio were significantly higher in ex-/current smokers compared
12
13 with non-smoker, while no significant correlation was observed between smoking status and
14
15 LDL-C level. In contrast, in statin group, there were no significant correlations between
16
17 smoking status and all any of these cholesterol parameters.
18
19

20
21
22 Conclusions:

23
24 We found that MDA-LDL level was affected by multiple factors, such as smoking status,
25
26 LDL-C level and male gender. The present findings give additional evidence that smoking
27
28 should be prohibited from MDA-LDL standpoint. Furthermore, statin therapy might have a
29
30 beneficial effect on the reduction of MDA-LDL level.
31
32

33
34 Trial registration:

35
36
37 N/A
38

39
40
41 **Main strengths**

42
43 Although oxidative LDL is associated with the marker of oxidative stress and the progression of
44
45 atherosclerosis, clinical factor which affects the oxidative LDL remains uncertain. Our study
46
47 revealed that MDA-LDL was associated with smoking and ~~the~~ MDA-LDL level would never
48
49 decrease with smoking cessation. However, MDA-LDL level was decreasing even in smokers
50
51 with statin therapy.
52
53
54

Formatted: Font: Not Bold

Formatted: Font: Times New Roman, 11 pt

Study limitations

Smoking cessation was not found to be effective for reducing MDA-LDL level in this study; however, favorable effects of smoking cessation would likely occur with regard to other parameters than MDA-LDL level. Thus smoking cessation is recommended at any time, even after long-term smoking, and is considered to provide cardiovascular health benefits.

This was a retrospective study, and the true effects of α -statin on MDA-LDL level remains uncertain. Finally, we did not examine the prognosis of the study population and therefore, the effects of smoking cessation and/or statin therapy remains uncertain, especially in terms of their impact on MDA-LDL level. Prospective studies should be required to obtain answers regarding their topics.

Introduction

The malondialdehyde modified low-density lipoprotein (MDA-LDL; oxidized LDL) is LDL that has been modified by MDA, leading to the production of a large amount of aldehyde when LDL becomes degenerated and oxidized¹.

It is known that MDA-LDL level is elevated in patients with dyslipidemia and diabetes mellitus (DM), both risks factors for atherosclerotic disease^{2,3}. Since MDA-LDL level has a positive correlation with the serum LDL level, the ratio of MDA-LDL/LDL-C (M/L) is used to evaluate the severity of oxidization of LDL; in some reports, not only MDA-LDL level, but also M/L ratio has been shown to increase in patients with DM compared with controls^{4,5}.

In patients with coronary artery disease (CAD), MDA-LDL level and M/L ratio have been shown to increase even when there are no other differences in the other lipid profiles⁶. In addition, it has been shown that the measurement of MDA-LDL level might be useful as a predictor of restenosis after percutaneous coronary intervention in patients with DM⁷. Based on these findings, it has been speculated that MDA-LDL level might be important marker of the progression of arteriosclerosis; however, the clinical factors possibly affecting MDA-LDL level have not been elucidated. Therefore, in the present study, we investigated “the clinical factors” affecting MDA-LDL level in high risk patients requiring catheter intervention.

Methods

Study patients

The study protocol (24-150[6916]) was approved by the ethics committee of the Jikei University School of Medicine. Six hundred consecutive patients who underwent cardiac catheterization from March 2010 to September 2011 were examined in this study. The baseline patient characteristics, including the clinical parameters and the biochemical data, were collected retrospectively from the hospital medical records. In addition, the results of the catheterization (i.e. the number of occluded or narrowed vessels), body weight, body mass index (BMI), coronary risk factors and medication profiles were also investigated. The patients taking eicosapentaenoic acid (EPA) were excluded since it has been demonstrated that EPA is major [lipid-loweringantihyperlipidemic](#) agents with potent antioxidant effects.

Data collection

Blood sampling was performed to examine the serum MDA-LDL, serum creatinine, hemoglobin A1c (HbA1c), B-type (brain) natriuretic peptide (BNP) and low-density lipoprotein cholesterol (LDL-C) levels. [The previous study reported by Tismikas et al.⁸ encompassed that PCI would affect the oxidative LDL level. To avoid the modification of LDL level by PCI, we tried to take a blood draw immediately before the cardiac catheterization or at the outpatient](#)

1
2
3
4
5
6
7
8
9 [clinic before admission](#). MDA-LDL level was measured by an ELISA using an anti-MDA-LDL
10
11 monoclonal antibody (ML25) and β -galactosidase anti-apoB monoclonal antibody (AB16)¹.[–]It
12
13 is well known that the combination of ML25 and AB16 can accurately detect MDA-LDL¹.
14

15
16 The concentration of MDA-LDL is defined at 1 mg/L of MDA-LDL produced artificially, which
17
18 shows the same signal as 1U/L of MDA-LDL in the serum. [Serum levels of LDL cholesterol](#)
19
20
21 [were determined enzymatically. \(Sekisui Medical Co., Ltd., Tokyo, Japan\)](#)
22
23

24 Diabetes mellitus (DM), hypertension, dyslipidemia and smoking were defined as described
25
26 previously^{9 10}. [Dyslipidemia was diagnosed with the use of lipid-lowering agents, the](#)
27
28 [presence of 1 or more of the following 3 lipid disorders at first fasting blood sampling or](#)
29
30 [both: a low-density lipoprotein \(LDL\) cholesterol level \$\geq\$ 140 mg/dL, a triglyceride level](#)
31
32 [\$\geq\$ 150 mg/dL, and a high-density lipoprotein \(HDL\) cholesterol level \$<\$ 40 mg/dL⁹. Blood](#)
33
34 [sampling was performed on the day of the catheter examination, except for the case that has](#)
35
36 [already been performed at outpatient clinic. Among 600 patients, 342 were performed on the](#)
37
38 [day of the catheter examination, and remaining 258 were at outpatient.](#)
39
40
41
42
43
44

45 The definition of smoking status ~~was~~ as follows: current smokers were those who were
46
47 smoking at the time of the study or who had smoked in the past year; the subjects who had quit
48
49 smoking more than one year before the study were defined as ex-smokers and those who had
50
51 never smoked were defined as non-smokers. Brinkman Index was used to evaluate the smoking
52
53
54

1
2
3
4
5
6
7
8
9 status of current/ex-smokers ¹¹.

10
11
12
13
14 *Statistical analysis*

15
16 Comparisons between MDA-LDL level and LDL-C level, age, BMI, HbA1c, Cr, BNP and
17
18 Brinkman index were performed with a linear regression analysis. Comparison between
19
20 Brinkman index and LDL-C was also performed with a linear regression analysis. Comparisons
21
22 of MDA-LDL levels between males and females, subjects with or without hypertension,
23
24 non-smokers versus ex-smokers versus current smokers, and among the various patient groups
25
26 after smoking cessation were performed with Mann-Whitney U test. [Kruskal Wallis test was](#)
27
28 [performed to evaluate the difference of MDA-LDL \(C\) and LDL-C \(D\) among 4 groups divided](#)
29
30 [by Brinkman index.](#) Multiple factors, which were considered to possibly modify MDA-LDL
31
32 levels were evaluated with a stepwise multiple regression analysis. Comparisons of LDL-C and
33
34 MDA-LDL level and M/L ratio between non-smokers and smokers were performed with
35
36 Mann-Whitney U test.

37
38
39
40
41
42
43
44
45 [A value of p <0.05 was considered to be statistically significant for all data that were](#)
46
47 [statistically analyzed using the SPSS software package, version 21.0 \(SPSS Inc., Chicago,](#)
48
49 [IL\). Statistical significance was defined as a value of p <0.05.](#)
50
51

Results

Baseline characteristics

The baseline characteristics of the patients in this study ~~were~~ shown in Table 1. The average age was 64.8 ± 11.4 years old and 80.3% of them were male. The percentages of non-smokers, ex-smokers and current smokers were 32%, 42% and 26%, respectively. The average LDL-C level and MDA-LDL level ~~were~~ 106.1 ± 30.8 mg/dl and 119.2 ± 48.7 U/L, respectively. The percentage of patients taking statin therapy was 55.3%. In addition, patient characteristics divided by smoking status into 3 groups (non-smokers, ex-smokers, current smokers) were shown in Table 1-3.

Table 1-1 Patient's Characteristics

	N n %	mean ± SD
Age, years		64.8 \pm 11.4
Male, gender (%)		80.3
Height, cm		165.4 \pm 25.1
Weight, kg		66.8 \pm 13.3
BMI, kg/m ²		24.4 \pm 3.74
Non-smoker, N n (%)	192 (32.4)	
Ex-smoker, N n (%)	247 (41.7)	
Current smoker, N n (%)	153 (25.8)	
Cr, mg/dL		1.38 \pm 1.94
eGFR, mL/min/1.73m ²		62.6 \pm 21.6
HbA1c, %		6.4 \pm 1.1
BNP, pg/mL		140 \pm 263
LDL-C, mg/dL		106.1 \pm 30.8
MDA-LDL, U/L		119.2 \pm 48.7
M/L		1.16 \pm 0.47

BMI: body mass index Cr: creatinine, eGFR: estimated glomerular filtration rate, BNP: B-type natriuretic peptide, LDL-C: low-density lipoprotein cholesterol, MDA-LDL: Malondialdehyde modified low density lipoprotein, M/L: Malondialdehyde modified low density lipoprotein/ low density lipoprotein cholesterol;

Table 1-2 Patient's Characteristics

Disease	N,n(%)
Diabetes Mellitus	252_(42.0)
Hypertension	455_(75.5)
Dyslipidemia	441_(73.5)
Medicine	N,n(%)
Sstatin	332_(55.3)
Ezetimibe	31_(5.2)
Fibrate	13_(2.1)
Coronary Artery Disease	N,n(%)
0VD	215_(35.8)
1VD	249_(41.5)
2VD	84_(14.0)
3VD	52_(8.7)

0VD: 0-vessel disease, 1VD: single-vessel disease, 2VD: double-vessel disease, 3VD: triple-vessel disease

Table 1-3 Patient's characteristics divided by smoking status

	Non-smoker	Ex-smoker	Current smoker
Number of patients (%)	192 (32.4)	250 (42.2)	151 (25.4)
Age	66.9 ± 12.4	65.5 ± 9.5	60.9 ± 12.2
Male, gender (%)	110 (57.3)	228 (91.2) *	138 (91.4)

<u>Height, cm</u>	<u>160.3 ± 11.0</u>	<u>166.0 ± 11.8*</u>	<u>167.4 ± 7.3</u>
<u>Weight, kg</u>	<u>62.7 ± 14.0</u>	<u>67.7 ± 10.9*</u>	<u>70.6 ± 14.8</u>
<u>BMI, kg/m²</u>	<u>24.2 ± 3.6</u>	<u>24.3 ± 3.0</u>	<u>25.0 ± 4.9</u>
<u>Cr, mg/dL</u>	<u>1.2 ± 1.5</u>	<u>1.6 ± 2.1</u>	<u>1.3 ± 2.1</u>
<u>HbA1c, %</u>	<u>6.3 ± 0.9</u>	<u>6.4 ± 1.1</u>	<u>6.4 ± 1.1</u>
<u>BNP, pg/mL</u>	<u>167.1 ± 302.0</u>	<u>133.2 ± 264.8</u>	<u>120.7 ± 201.1</u>
<u>LDL-C, mg/dL</u>	<u>102.3 ± 29.1</u>	<u>104.2 ± 28.4</u>	<u>114.8 ± 35.2**</u>
<u>MDA-LDL, U/L</u>	<u>108.0 ± 43.6</u>	<u>122.1 ± 49.2*</u>	<u>129.3 ± 52.1</u>
<u>M/L</u>	<u>2.06 ± 0.85</u>	<u>2.26 ± 0.82</u>	<u>2.6 ± 0.99</u>
<u>LVEF, %</u>	<u>60.2 ± 10.0</u>	<u>56.7 ± 9.6*</u>	<u>54.4 ± 12.1</u>
<u>Diabetes Mellitus</u>	<u>72 (37.5)</u>	<u>112 (44.8)</u>	<u>65 (43.0) *</u>
<u>Hypertension</u>	<u>155 (80.7)</u>	<u>191 (76.4)</u>	<u>104 (68.9) *</u>
<u>Dyslipidemia</u>	<u>134 (69.8)</u>	<u>185 (74.0)</u>	<u>116 (76.8)</u>
<u>Statin</u>	<u>111 (57.8)</u>	<u>146 (58.4)</u>	<u>73 (48.3)</u>
<u>Ezetimibe</u>	<u>10 (3.6)</u>	<u>14 (5.6)</u>	<u>7 (4.6)</u>
<u>Fibrate</u>	<u>2 (1.0)</u>	<u>6 (2.4)</u>	<u>5 (3.3)</u>
<u>Coronary Artery Disease</u>			
<u>0VD</u>	<u>82 (42.7)</u>	<u>83 (33.2)</u>	<u>49 (32.5)</u>
<u>1VD</u>	<u>69(35.9)</u>	<u>105(42.0)</u>	<u>71(47.0) *</u>
<u>2VD</u>	<u>26 (13.5)</u>	<u>34 (13.6)</u>	<u>22 (14.6)</u>
<u>3VD</u>	<u>15 (7.8)</u>	<u>28 (7.2)</u>	<u>9 (6.0)</u>
—	Non-smoker	Ex-smoker	Current smoker
Number of			
patients (%)	192 (32.4)	250 (42.2)	151 (25.4)

Age	66.9 ± 12.4	65.5 ± 9.5	60.9 ± 12.2
Male, gender (%)	110 (57.3)	228 (91.2)*	138 (91.4)
Height, cm	160.3 ± 11.0	166.0 ± 11.8*	167.4 ± 7.3
Weight, kg	62.7 ± 14.0	67.7 ± 10.9*	70.6 ± 14.8
BMI, kg/m ²	24.2 ± 3.6	24.3 ± 3.0	25.0 ± 4.9
Cr, mg/dL	1.2 ± 1.5	1.6 ± 2.1	1.3 ± 2.1
HbA1c, %	6.3 ± 0.9	6.4 ± 1.1	6.4 ± 1.1
BNP, pg/mL	167.1 ± 302.0	133.2 ± 264.8	120.7 ± 201.1
LDL-C, mg/dL	102.3 ± 29.1	104.2 ± 28.4	114.8 ± 35.2**
MDA-LDL, U/L	108.0 ± 43.6	122.1 ± 49.2*	129.3 ± 52.1
M/L	2.06 ± 0.85	2.26 ± 0.82	2.6 ± 0.99
LVEF, %	60.2 ± 10.0	56.7 ± 9.6*	54.4 ± 12.1
Diabetes Mellitus	72 (37.5)	112 (44.8)	65 (43.0)*
Hypertension	155 (80.7)	191 (76.4)	104 (68.9)*
Dyslipidemia	134 (69.8)	185 (74.0)	116 (76.8)
Statin	111 (57.8)	146 (58.4)	73 (48.3)
Ezetimibe	10 (3.6)	14 (5.6)	7 (4.6)
Fibrate	2 (1.0)	6 (2.4)	5 (3.3)

~~Coronary Artery~~~~Disease~~

0VD	82 (42.7)	83 (33.2)	49 (32.5)
1VD	69 (35.9)	105 (42.0)	71 (47.0) *
2VD	26 (13.5)	34 (42.0)	22 (14.6)
3VD	15 (7.8)	28 (7.2)	9 (6.0)

~~*: P <0.05 vs. Non-smoker, **: P <0.05 vs. Ex-smoker~~

Clinical factors affecting MDA-LDL level

To elucidate the determinants of MDA-LDL level, ~~we first performed~~ a simple regression analysis ~~was performed~~. (Fig. 1). MDA-LDL level showed a significantly positive correlation with LDL-C level (Fig. 1A) and a negative correlation with age (Fig. 1B). In addition, MDA-LDL levels ~~was~~ were significantly higher in males as well as ~~the~~ patients without hypertension (Figs. 1C, D). BMI, HbA1c, Cr and BNP level had no impact on MDA-LDL level (Figs. 1E-H).

Correlation of smoking status with MDA-LDL level

1
2
3
4
5
6
7
8
9 ~~N~~We next, ~~examined~~ the impact of smoking status on MDA-LDL level was performed.
10
11 MDA-LDL, but not LDL-C, showed a significantly positive correlation with the smoking
12
13 profiles indicated by Brinkman index (*Fig. 2-1-A, B*). However, considering the bias of the
14 Brinkman distribution, in addition to Fig. 2-1-A, B, MDA-LDL level and LDL-C level were
15 analyzed with dividing by Brinkman index into 4 groups. There was a significant difference of
16 MDA-LDL level between non-smoker and current-smoker, whereas there was no significant
17 difference of LDL-C regardless of the smoking status (*Fig. 2-1C, D*).

Formatted: Font: Italic

Formatted: Font: Italic

26
27 The patients were subsequently divided into three groups according to their smoking status:
28
29 Non-, ex- and current smokers. MDA-LDL level was significantly higher in both ex- and
30
31 current smokers compared to that in non-smokers (*Fig. 2-C2-E*). Moreover, when patients were
32
33 compared based on the number of years after smoking cessation, not only ~~the~~ current smoking
34
35 group, but also ~~the~~ group that had quit smoking within 10 years and that where the patients had
36
37 quit smoking more than 21 years earlier showed higher MDA-LDL levels than ~~did the~~
38
39 non-smoking group (*Fig. 2-2-FD*).

44 ~~This suggests that MDA-LDL level will never completely recover once a subject has started~~
45 ~~smoking~~.

Formatted: Font: Italic, Not Strikethrough

52 *Clinical factors affecting MDA-LDL level identified in multiple regression analysis*

To assess the independent determinants of MDA-LDL level, a multiple regression analysis was performed. After removing the confounding factors, MDA-LDL level was shown to be positively correlated with LDL-C level ($p < 0.001$), Brinkman index ($p = 0.009$) and a male gender ($p = 0.019$) (Table 2).

Table 2 Multiple Regression Analysis

Significant variable	Regression coefficients	Standard error	Standard regression coefficients	F	p
LDL-C	0.675	0.06	0.429	128.089	<0.001
Brinkman index	0.008	0.003	0.105	71.502	0.009
Gender	11.511	4.908	0.94	49.901	0.019

Objective variable: MDA-LDL

Explanatory variable: BMI, Age, Gender, brinkman index, Cr, BNP, LDL-C, HbA1c, HT

No significant variables: BMI, Age, Cr, BNP, HbA1c, HT

Effects of statin therapy on the correlations of smoking status with MDA-LDL or LDL-cholesterol level

The correlations of ~~the~~ smoking status with MDA-LDL/LDL-C level ~~was~~ ~~ere~~ investigated after patients were divided into two groups; those with or without statin treatment (*Fig.-3*). In non-statin-treated group, MDA-LDL level as well as MDA-LDL/LDL-C ratio, was significantly increased in ex-/current smokers compared to those in non-smokers, although there was no

1
2
3
4
5
6
7
8
9 | significant differences in LDL-C levels between the subjects with the different smoking status.

10
11 | LDL-C level was not significantly different in non-statin-treated and statin-treated groups.

16 17 **Discussion**

18
19 | In this study, we investigated the factors associated with MDA-LDL level in high risk patients
20
21 | requiring cardiac catheterization. According to a multivariate analysis, Brinkman index, as well
22
23 | as the LDL-C level and gender were found to be significantly associated with MDA-LDL level.

24
25 | Furthermore, we found that smoking cessation was not effective for reducing MDA-LDL level,
26
27 | even after the patients had quit smoking for many years. – However, we found evidence that
28
29 | statin treatment may reduce MDA-LDL level, which could possibly help in the treatment of
30
31 | smokers.

32
33 | It has been reported that smoking may affect susceptibility of plasma LDL to peroxidative
34
35 | modification. Modified LDL has been shown to be the preferred substrate for macrophages and
36
37 | induces their subsequent transformation into foam cells^{12 13}. Thus, oxidative stress is very
38
39 | important for synthesis of modified-LDL. However, it is noteworthy that MDA-LDL level was
40
41 | emphatically influenced by smoking but not by obesity (estimated by BMI), hypertension,
42
43 | diabetes mellitus (estimated by HbA1c level), renal failure (estimated by Cr level) or heart
44
45 | failure (estimated by BNP level) by the multivariate analysis in this study, though all of these
46
47
48
49
50
51
52
53
54

1
2
3
4
5
6
7
8
9 clinical characteristics have been shown to increase oxidative stress. The precise mechanisms by
10 which smoking increases MDA-LDL level remains unclear at present.

11
12
13
14 The current study clearly showed that smoking was substantially harmful with regard to
15 increasing MDA-LDL level. When we examined the effect of smoking status on MDA-LDL
16 level among ~~the~~ non-smokers, ex-smokers and current smokers, we found that MDA-LDL level
17 was still higher in ~~the~~ ex-smokers than in ~~the~~ non-smokers and was unexpectedly similar to the
18 level in ~~the~~ current smokers. Furthermore, we examined the effects of the period of smoking
19 cessation and the analysis indicated that even many years after smoking cessation, there was no
20 significant reduction of MDA-LDL level. This result suggests that smoking should never be
21 started, and that if started, it is important to quit smoking as soon as possible before becoming a
22 heavy smokers.
23
24
25
26
27
28
29
30
31
32
33
34
35
36

37 The present results suggests that smoking keeps MDA-LDL level elevated for a long time.
38
39 Therefore, a method for lowering MDA-LDL level is especially needed for smokers. One
40 possible candidate ~~might~~would be statin therapy. Statins facilitate the LDL uptake in
41 hepatocytes, decrease old LDL (which ~~is~~ easily oxidized), and thereby reduce the risk of LDL
42 oxidization¹⁴. The decreases in fatty acids and cholesterol in the lipoprotein are also likely to
43 lead to a decrease in oxidization¹⁵. In the current study, we examined the effects of statin
44 treatment on MDA-LDL levels between non-smokers and smokers. MDA-LDL level was found
45
46
47
48
49
50
51
52
53
54

1
2
3
4
5
6
7
8
9 to be significantly higher in smokers than in non-smokers in the stain (-) group. On the other
10
11 hand, the effect of smoking on LDL-C level was not seen in the statin (+) group, the levels
12
13 ~~wasere~~ similar between smokers and non-smokers. The ratio of MDA-LDL/LDL-C showed a
14
15 similar result. These results may suggest that statin therapy ~~wouldcan~~ reduces MDA-LDL level
16
17
18 in smokers to a level similar to that in non-smokers.

19
20
21 It has been reported that cigarette smoking is one of the risk factors for organic stenosis but that
22
23 it does not act alone in contributing to the progression of atherosclerosis ^{15 16}. In addition,
24
25
26 atherosclerosis was not produced by smoking alone in animal models ¹⁷. However, cigarette
27
28 smoking acts in concert with other risk factors such as hypercholesterolemia to accelerate
29
30 atherosclerosis^{15 16 18 19 20}. The current study is in agreement with the previous reports.
31
32
33 Importantly, it has been shown that among other risk factors cigarette smoking alone is a highly
34
35 significant risk factor for coronary spasm ^{21 22 23 24 25} and treatment with fluvastatin reduced the
36
37 coronary spasm ²⁶. Thus, smoking cessation combined with statin therapy would be beneficial
38
39
40 for prevention of ischemic heart disease by reducing progression of atherosclerosis and
41
42
43 suppressing coronary spasm.
44
45

46
47 In the present study, we performed multivariate analysis for determination of MDA-LDL by
48
49 using the factors of LDL-C level, age, BMI, HbA1c, Cr, BNP and Brinkman index; however,
50
51 there may be other factors associated with MDA-LDL in a direct or indirect manner. As a matter
52
53
54

1
2
3
4
5
6
7
8
9 of fact, the previous study by Matsuda et al. showed that MDA-LDL level was correlated with
10 triglyceride, HDL-C, metformin and α -glucosidase inhibitors in statin-treated diabetes patients
11 with CAD²⁷.

12
13
14
15
16 Then, we performed another multivariate analysis. As a result, it revealed that MDA-LDL level
17 was correlated with LDL-C, triglyceride and smoking, however, not with HDL-C,
18 anti-hypertensive drugs, anti-diabetes agents such as metformin and α -glucosidase inhibitors,
19 number of the vessels with CAD and CRP. (Precise data not shown) Furthermore, there may be
20 confounding factors among them; and the correlation among each factors were also investigated.
21
22 As a result, there were only slight correlation between CAD and HDL-C ($R=-0.163$), between
23 CAD and smoking ($R=0.098$), between triglyceride and HDL-C ($R=0.203$) and between
24 triglyceride and smoking ($R=-0.163$) (Precise data not shown). The reason of the difference
25 between the previous study and ours may be due to the different study population. In any case, it
26 would be safe to say that smoking affected MDA-LDL level in a fairly direct manner.
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

45 **Conclusion**

46
47 We found that MDA-LDL level was affected by multiple factors such as smoking status (as
48 indicated by Brinkman index), LDL-C level and gender. In addition to its other health effects,
49 smoking should be strongly prohibited due to its harmful effects from a MDA-LDL standpoint.
50
51
52
53
54

1
2
3
4
5
6
7
8
9 We recommend that patients should never smoke, but that once smoking has started, it is
10
11 essential to quit smoking as early as possible and to cut back on the number of cigarettes
12
13 consumed. Furthermore, statin therapy might have a beneficial effect on the reduction of
14
15 MDA-LDL level.
16
17
18
19
20
21

22 Footnotes

23
24 Contributors: Conceived and designed the experiments: KO TT KM MY. Performed the

25
26 experiments: KO HS SA TN TO. Performed the statistical analysis: KO HS KM MY.

27
28 Contributed reagents/materials/analysis tools: KO TT TN KM. Wrote the paper: KO TT KM

29
30
31 MY.

32
33
34
35 -

36
37
38 -

39
40 Competing interests: None

41
42
43 -

44
45 Funding: None

46
47
48 -

49
50 Ethics approval: The study protocol (24-150[6916]) was approved by the ethics committee of
51
52 the Jikei University School of Medicine.
53
54

[Provenance and peer review: Not commissioned; externally peer reviewed](#)

[Data sharing: No additional data are available.](#)

References

1. Kotani K, Maekawa M, Kanno T, et al. Distribution of immunoreactive malondialdehyde-modified low-density lipoprotein in human serum. *Biochimica et biophysica acta* 1994;1215(1-2):121-5.
2. Kitano S, Kanno T, Maekawa M, et al. Improved method for the immunological detection of malondialdehyde-modified low-density lipoproteins in human serum. *Analytica Chimica Acta* 2004;509(2):229-35.
3. Kondo A, Manabe M, Saito K, et al. Insulin treatment prevents LDL from accelerated oxidation in patients with diabetes. *Journal of atherosclerosis and thrombosis* 2002;9(6):280-7.
4. Kondo A, Muranaka Y, Ohta I, et al. Relationship between triglyceride concentrations and LDL size evaluated by malondialdehyde-modified LDL. *Clinical chemistry* 2001;47(5):893-900.
5. Kondo A, Li J, Manabe M, et al. Relationship between high-density lipoprotein-cholesterol and malondialdehyde-modified low-density lipoprotein concentrations. *Journal of atherosclerosis and thrombosis* 2003;10(2):72-8.
6. Tanaga K, Bujo H, Inoue M, et al. Increased circulating malondialdehyde-modified LDL levels in patients with coronary artery diseases and their association with peak sizes of LDL particles. *Arteriosclerosis, thrombosis, and vascular biology* 2002;22(4):662-6.
7. Shigematsu S, Takahashi N, Hara M, et al. Increased incidence of coronary in-stent restenosis in type 2 diabetic patients is related to elevated serum malondialdehyde-modified low-density lipoprotein. *Circulation journal : official journal of the Japanese Circulation Society* 2007;71(11):1697-702.
8. Tsimikas S, Lau HK, Han KR, et al. Percutaneous coronary intervention results in acute increases in oxidized phospholipids and lipoprotein(a): short-term and long-term immunologic responses to oxidized low-density lipoprotein. *Circulation* 2004;109(25):3164-70.
9. Yagi H, Komukai K, Hashimoto K, et al. Difference in risk factors between acute coronary syndrome

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

- and stable angina pectoris in the Japanese: smoking as a crucial risk factor of acute coronary syndrome. *Journal of cardiology* 2010;55(3):345-53.
10. Sekiyama H, Nagoshi T, Komukai K, et al. Transient decrease in serum potassium level during ischemic attack of acute coronary syndrome: paradoxical contribution of plasma glucose level and glycohemoglobin. *Cardiovascular diabetology* 2013;12:4.
 11. Brinkman GL, Coates EO, Jr. The effect of bronchitis, smoking, and occupation on ventilation. *The American review of respiratory disease* 1963;87:684-93.
 12. Yokode M, Kita T, Arai H, et al. Cholesteryl ester accumulation in macrophages incubated with low density lipoprotein pretreated with cigarette smoke extract. *Proceedings of the National Academy of Sciences of the United States of America* 1988;85(7):2344-8.
 13. Harats D, Ben-Naim M, Dabach Y, et al. Cigarette smoking renders LDL susceptible to peroxidative modification and enhanced metabolism by macrophages. *Atherosclerosis* 1989;79(2-3):245-52.
 14. Gotto AM. Interactions of the major risk factors for coronary heart disease. *The American Journal of Medicine* 1986;80(2):48-55.
 15. Willett WC, Green A, Stampfer MJ, et al. Relative and absolute excess risks of coronary heart disease among women who smoke cigarettes. *The New England journal of medicine* 1987;317(21):1303-9.
 16. Stein Y, Harats D, Stein O. Why is smoking a major risk factor for coronary heart disease in hyperlipidemic subjects? *Annals of the New York Academy of Sciences* 1993;686:66-9; discussion 69-71.
 17. Asano M, Ohkubo C, Hirokawa A, et al. Smoking Research Foundation Annual Research Report, On macro- and microcirculatory effects of tobacco smoke inhalation on atherogenesis in the rabbit. 1987:251-61.
 18. Anderson KM, Wilson PW, Odell PM, et al. An updated coronary risk profile. A statement for health professionals. *Circulation* 1991;83(1):356-62.
 19. Gotto AM, Jr. Interactions of the major risk factors for coronary heart disease. *Am J Med* 1986;80(2a):48-55.
 20. Holbrook JH, Grundy SM, Hennekens CH, et al. Cigarette smoking and cardiovascular diseases. A statement for health professionals by a task force appointed by the steering committee of the American Heart Association. *Circulation* 1984;70(6):1114a-17a.
 21. Caralis DG, Deligonul U, Kern MJ, et al. Smoking is a risk factor for coronary spasm in young women. *Circulation* 1992;85(3):905-9.
 22. Sugiishi M, Takatsu F. Cigarette smoking is a major risk factor for coronary spasm. *Circulation* 1993;87(1):76-9.
 23. Yoshimura M, Yasue H, Nakayama M, et al. A missense Glu298Asp variant in the endothelial nitric oxide synthase gene is associated with coronary spasm in the Japanese. *Human genetics*

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1998;103(1):65-9.

24. Nakayama M, Yasue H, Yoshimura M, et al. T-786-->C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. *Circulation* 1999;99(22):2864-70.

25. Takaoka K, Yoshimura M, Ogawa H, et al. Comparison of the risk factors for coronary artery spasm with those for organic stenosis in a Japanese population: role of cigarette smoking. *International journal of cardiology* 2000;72(2):121-6.

26. Yasue H, Mizuno Y, Harada E, et al. Effects of a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, fluvastatin, on coronary spasm after withdrawal of calcium-channel blockers. *Journal of the American College of Cardiology* 2008;51(18):1742-8.

27. Matsuda M, Tamura R, Kanno K, et al. Impact of dyslipidemic components of metabolic syndrome, adiponectin levels, and anti-diabetes medications on malondialdehyde-modified low-density lipoprotein levels in statin-treated diabetes patients with coronary artery disease. *Diabetology & metabolic syndrome* 2013;5(1):77.

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Not Bold

Formatted: Font: Times New Roman

Formatted: Font: Times New Roman, Font color: Red

Formatted: Font: Times New Roman, Not Bold, Font color: Red

Formatted: Font: Times New Roman, Font color: Red

Formatted: Font: Not Bold

1
2
3
4
5
6
7
8 **Figure legends**
9

10 *Fig. 1 Correlations of MDA-LDL level with various clinical factors*

11
12 The clinical factors affecting MDA-LDL level are shown in (A)-(H).
13
14

15
16
17
18 *Fig. 2 Correlation of smoking status with MDA-LDL and LDL-cholesterol levels*

19
20 Correlation between MDA-LDL level and Brinkman index (A) and, between LDL-C level and
21
22 Brinkman index (B) was determined with a linear regression analysis.
23

24
25 Kruskal Wallis test was performed to evaluate the difference of MDA-LDL (C) and LDL-C (D)
26 among 4 groups divided by Brinkman index. Comparison of MDA-LDL levels among
27 non-smokers, ex-smokers and current smokers (E). Comparison of MDA-LDL levels in each
28 patient group among non-smokers and ex-smokers who had quit more than 21 years earlier,
29 ex-smokers who had quit 11 to 20 years ago, ex-smokers who had quit less than 10 years ago
30 and current smokers (F).
31
32

33
34 ~~Comparison of MDA-LDL levels among non-smokers, ex-smokers and current smokers (C).~~
35
36 ~~Comparison of MDA-LDL levels in each patient group among non-smokers and ex-smokers~~
37 ~~who had quit more than 21 years earlier, ex-smokers who had quit 11 to 20 years ago,~~
38 ~~ex-smokers who had quit less than 10 years ago and current smokers (D).~~
39
40
41
42
43
44
45
46
47
48
49

50
51
52
53
54 *Fig. 3 Effects of the statin therapy on the correlations of smoking status with MDA-LDL or*

1
2
3
4
5
6
7
8
9 *LDL-cholesterol level*

10
11 The correlations of the smoking status with MDA-LDL and LDL-C levels were investigated
12
13
14 after dividing the patients into two groups; with (A) or without (B) statin treatment.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41

42 **Table 2**
43 **Multipl**
44 **e**
45 **Regress**
46 **ion**
47 **Analysi**
48 **s**
49
50
51
52
53
54

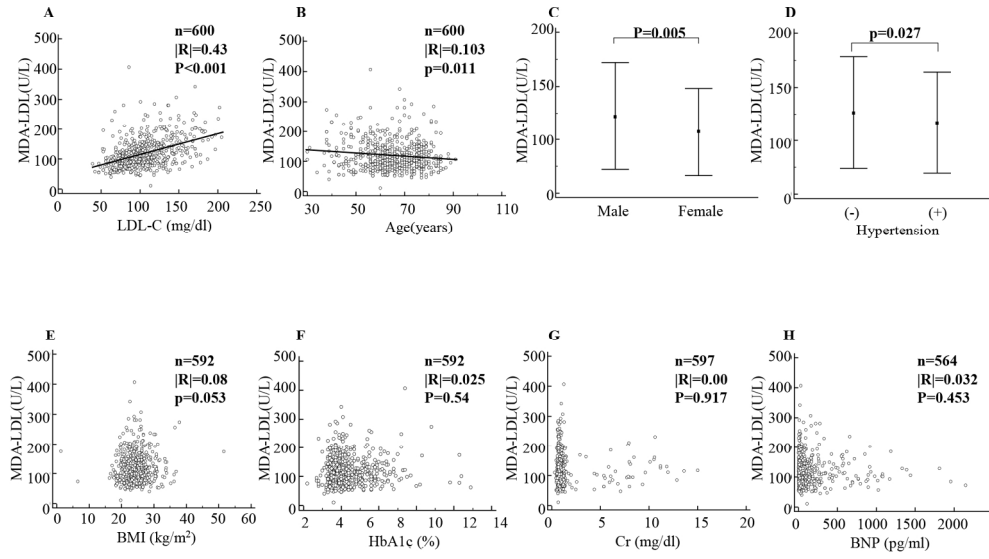


Figure 1

173x130mm (300 x 300 DPI)

Review only

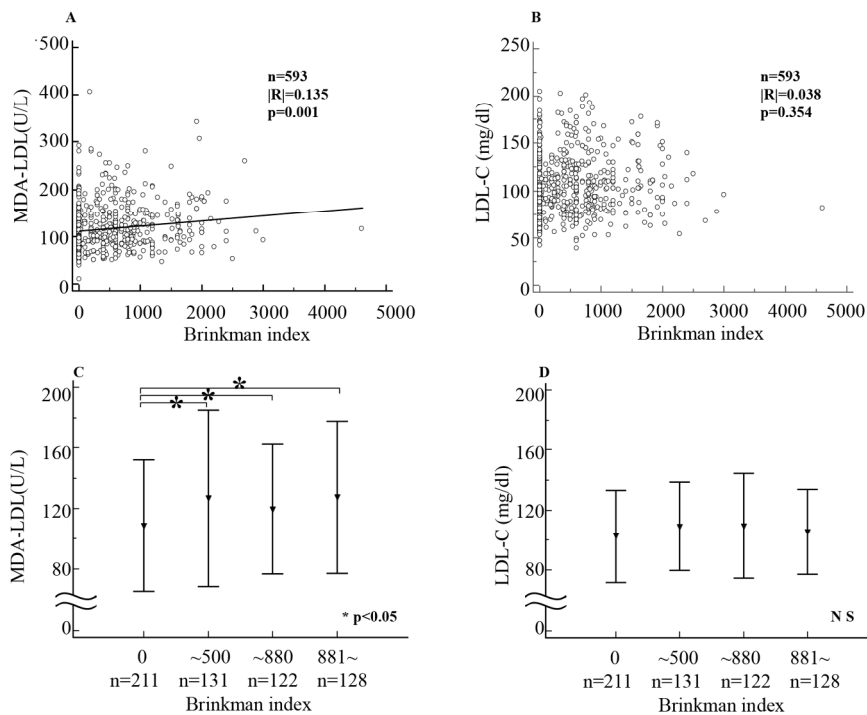


Figure 2-1

173x130mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

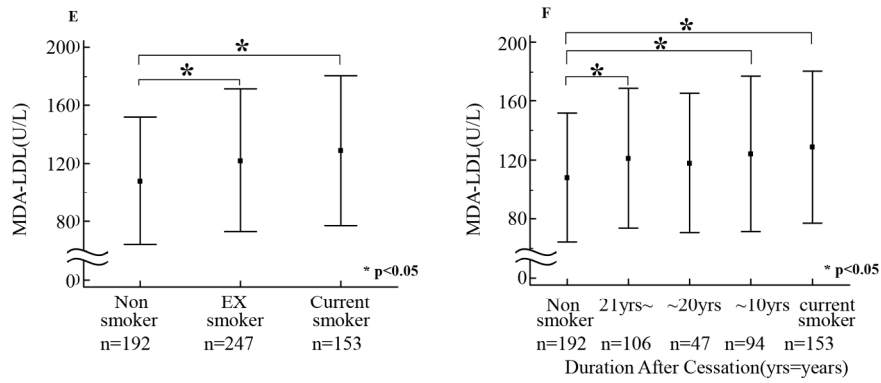


Figure 2-2

173x130mm (300 x 300 DPI)

View only

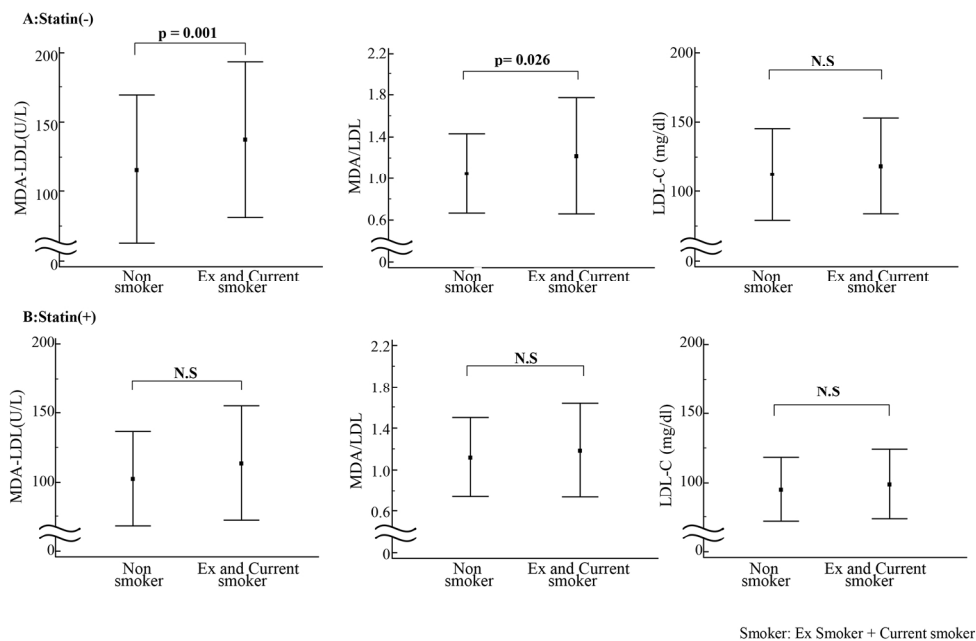


Figure 3

173x130mm (300 x 300 DPI)