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Cardiovascular risk assessment using LOX-index and Self-Rating Depression Scale

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ABSTRACT

Objective: LOX-Index is a novel biomarker for cardiovascular disease (CVD) and is calculated by multiplying LOX-1 ligands containing apolipoprotein B (LAB) and soluble LOX-1 (sLOX-1). The Framingham risk score (FRS) is a common clinical tool for risk assessment of coronary artery disease. Mental stress can also be an important risk factor for CVD. The purpose of this study was to examine the relationship between LOX-Index and FRS or mental stress.

Methods: LOX-Index was measured in 453 subjects including 150 consecutive outpatients with lifestyle-related diseases such as diabetes, hyperlipidemia, and hypertension and 303 healthy volunteers. Mental stress was evaluated by the Self-Rating Depression Scale (SDS).

Results: LOX-Index was significantly related with the 10-years risk of FRS. Multiple regression analysis demonstrated that LAB was closely associated with the smoking status, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). There were no significant associations between LOX-Index and the SDS scores; however, by simultaneously using LOX-Index and SDS, the subjects could be classified in terms of oxidative stress and mental stress.

Conclusions: LOX-Index appears to be a comprehensive marker that could evaluate the status of multiple CVD risk factors. The classification with LOX-Index and SDS could contribute to the risk assessment for CVD.

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1. Introduction

Atherosclerosis-based cardiovascular disease (CVD) is still a major cause of death in developed countries, including Japan. Furthermore, *Karoshi*, death from over-work, is a pressing societal issue in Japan and it is usually the extreme result of CVD. The pathogenesis of CVD is a complicated process; however, recent research has revealed that responses to various types of stress including mental stress and oxidative stress play an important role in the pathogenesis of these diseases. There has been growing accumulating evidences that well-known atherosclerotic risk factors such as hypertension, diabetes, hyperlipidemia, and smoking can induce oxidative stress in the cardiovascular system. Under enhanced oxidative stress, low-density lipoprotein (LDL) is oxidatively modified. The formed oxidized LDL induces various biological activities that are related to atherosclerotic processes. Oxidized LDL causes endothelial dysfunction, promotes the proliferation of vascular smooth muscle cells, and induces the expression of adhesion molecules and chemokines [1–3]. These pathophysiological effects are mainly mediated via LOX-1 [4,5]. Recently, the Suita cohort investigation has revealed that a higher LOX-index, in which the LOX-1 ligands containing apolipoprotein B (LAB) and soluble LOX-1 (sLOX-1) were multiplied, was associated with an increased risk of CVD and stroke [6]. Thus, LOX-Index might be a novel predictive marker for these diseases from the standpoint of oxidative stress.

There have been numerous studies that have indicated an etiological association between mental stress and the development of CVD [7]. The mechanisms by which mental stress or depression induces and exacerbates CVD remain unclear; however, the sympathetic nervous system or the hypothalamic–pituitary–adrenal axis might be involved. Recent research has indicated that mental exacerbation might be associated with the oxidative stress. For example, the production of reactive oxygen species (ROS) has been enhanced under high mental stress in both animals and humans. Depressive symptoms have been correlated with lipid peroxidation in human blood [8]. Andersson et al. investigated the effects of psychological stress on LOX-1 expression in rats [9]. Psychological stress upregulated LOX-1 levels in the vessel wall in by psychological stress through the formation of ROS. Their experimental observations suggested the possibility that LOX-1 might be a key molecule that linked inking oxidative stress and mental stress.

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The Framingham risk score (FRS) is a simplified and common clinical tool for the assessment of the risk for coronary artery disease as well as in the identification of individuals who were candidates for risk factors modifications [10]. The FRS is based on sex and age stratified tables with specific scores assigned for LDL and high-density lipoprotein (HDL) cholesterol levels, smoking status, and systolic blood pressure. In the present study, the relationship between LOX-Index and the FRS was examined in outpatients with lifestyle-related diseases such as diabetes, hyperlipidemia, and hypertension and in healthy volunteers. Furthermore, we also examined the relationship of LOX-Index and mental stress as assessed by the Self-Rating Depression Scale (SDS).

2. Method

2.1. Subjects

Between May 2014 and June 2015, 453 subjects including 150 consecutive outpatients with lifestyle-related diseases and 303 healthy volunteers were recruited for the present study. The purpose of the present study was explained to the participants in the documents, and written informed consent was obtained from all participants. The present study was approved by the ethics committee of Kobe Rosai Hospital.

All enrolled patients were interviewed and clinically examined. Demographic information (age and sex) and medical history were recorded. Hypertension was defined as a systolic pressure ≥140 mm Hg or a diastolic pressure ≥90 mm Hg, or if antihypertensive drugs were used. Dyslipidemia was defined as plasma LDL ≥ 140 mg/dL, plasma triglycerides (TG) ≥ 150 mg/dL, or plasma HDL < 40 mg/dL or if lipid-lowering drugs were used. Diabetes mellitus was defined as previous or current plasma fasting glucose ≥126 mg/dl or if hypoglycemic agents were used.

2.2. Measurement of LOX-Index

All measurements of LOX-Index were performed at NK Medico Co (Tokyo, Japan) similar to the previous investigation [6,11]. In brief, the recombinant LOX-1 was immobilized on plates, and the serum LAB levels were measured by sandwich chemiluminescent enzyme immunoassay (CLEIA) using recombinant sLOX-1 and monoclonal antibody against the extracellular domain of apolipoprotein (ApoB), that is, a chicken monoclonal anti-human ApoB antibody HUC20. This assay system can measure the levels of LAB in the serum, such as VLDL remnants or oxidized LDL [12]. The plasma levels of sLOX-1 were measured by sandwich CLEIA using two kinds of monoclonal antibodies against the extracellular domain of LOX-1, that is, B017M and a chicken monoclonal anti-human LOX-1 antibody HUC3–48.

2.3. Evaluation of depression by the Self-Rating Depression Scale

The SDS designed by Zung was used to quantify the depression level who had experienced depression-related symptoms [12]. Among the enrolled subjects, 331 participants agreed to the evaluation of their mental status using SDS. The SDS included 10 positively worded items and 10 negatively worded items that assess the symptoms of depression. The item responses were rated from 1 to 4, and higher scores correspond to more frequent symptoms. Therefore, for each item, patients give a score according to whether the item has occurred: 1 = never/very rarely/rarely; 2 = once in a while/some of the time/occasionally; 3 = relatively often/very often/often; 4 = most of the time/always/almost always. The SDS scores were used to define the following four categories of depression severity: within the normal range (below 40 points); presence of minimal to mild depression (40–47 points); presence of moderate to marked depression (48–55 points); and presence of severe to extreme depression (56 points and above). In the present study, the subjects who had scores over 40 points were defined as being depressed.

2.4. Calculation of the Framingham risk score

The FRS is based on sex- and age-stratified tables with specific scores assigned for total and HDL cholesterol levels, smoking status, and systolic blood pressure (untreated and treated) and also provides an estimate for the 10-year risk of developing cardiovascular disease [11]. The FRS was calculated using a computer program, which took age, sex, LDL-cholesterol, HDL cholesterol, systolic and diastolic blood pressure, smoking and the presence of diabetes into account.

2.5. Statistical analysis

The continuous data are described as the mean and standard deviation (SD). The categorical variables are displayed as number (percentage). The LAB, sLOX-1 and TG levels were log-transformed for all of the regression analyses. Multiple linear regression analyses were used to explore the relationships between variables. Standardized coefficient and 95% confidence interval (CI) were calculated.

To evaluate the collinearity between variables, the variance inflation factor (VIF) was estimated. If the estimated VIF for one variable is over 10, there is strong possibility of the existence of collinearity. The statistical analyses were performed using IBM SPSS Statistics Version 22 or the GraphPad Prism version 5. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Association of LOX-Index with FRS

A total of 453 subjects were enrolled in this study, and their characteristics are shown in Table 1. Fig. 1 is a histogram for LOX-Index and log-converted LOX-Index in men and women. The LOX-Index in the enrolled subjects was distributed from 310 to 3728 in a wide range. After log conversion, LOX-Index had normal distribution (Kolmogorov–Smirnov test: $p < 0.001$ for LOX-Index and $p = 0.200$ for log-converted LOX-Index).

The relationship between the 10-year risk of FRS and the log-converted LOX-Index was examined in these subjects. As shown in Fig. 2, there were significant positive correlation between the 10-year risk of FRS and the log-converted LOX-Index in the male and female populations. FRS is determined by age, sex, LDL, HDL, TG, the smoking status, and the presence of hypertension, and diabetes; therefore, the standard multiple linear regression analyses for LAB were performed using these factors as independent variables to evaluate the most influential factors for LAB and sLOX-1. As shown in Table 2, among these variables, the current smoking status and LDL were positively associated with LAB, and HDL was negatively associated with LAB. The goodness of fit for the regression models was significant in the multiple linear regression analysis although adjusted the R2 of the regression model was 14.5%. The estimated VIFs indicated that there was little evidence for the existence of collinearity. On the other hand, sLOX-1 was not associated with any of these variables except the presence of DM.

Table 1
Patient characteristics.

Patients characteristics		
Age, mean (SD), y	54.1	9.85
Male sex, no. (%)	257	56.7
Diabetes no. (%)	54	11.9
Hypertension no. (%)	114	25.2
Hyperlipidemia no. (%)	115	25.4
Current smokers no. (%)	51	11.3
LOX-Index, mean (SD)	985.0	420.5
sLOX-1, mean (SD), μg/mL	351.0	119.6
LAB, mean (SD), μg/mL	2.83	0.76

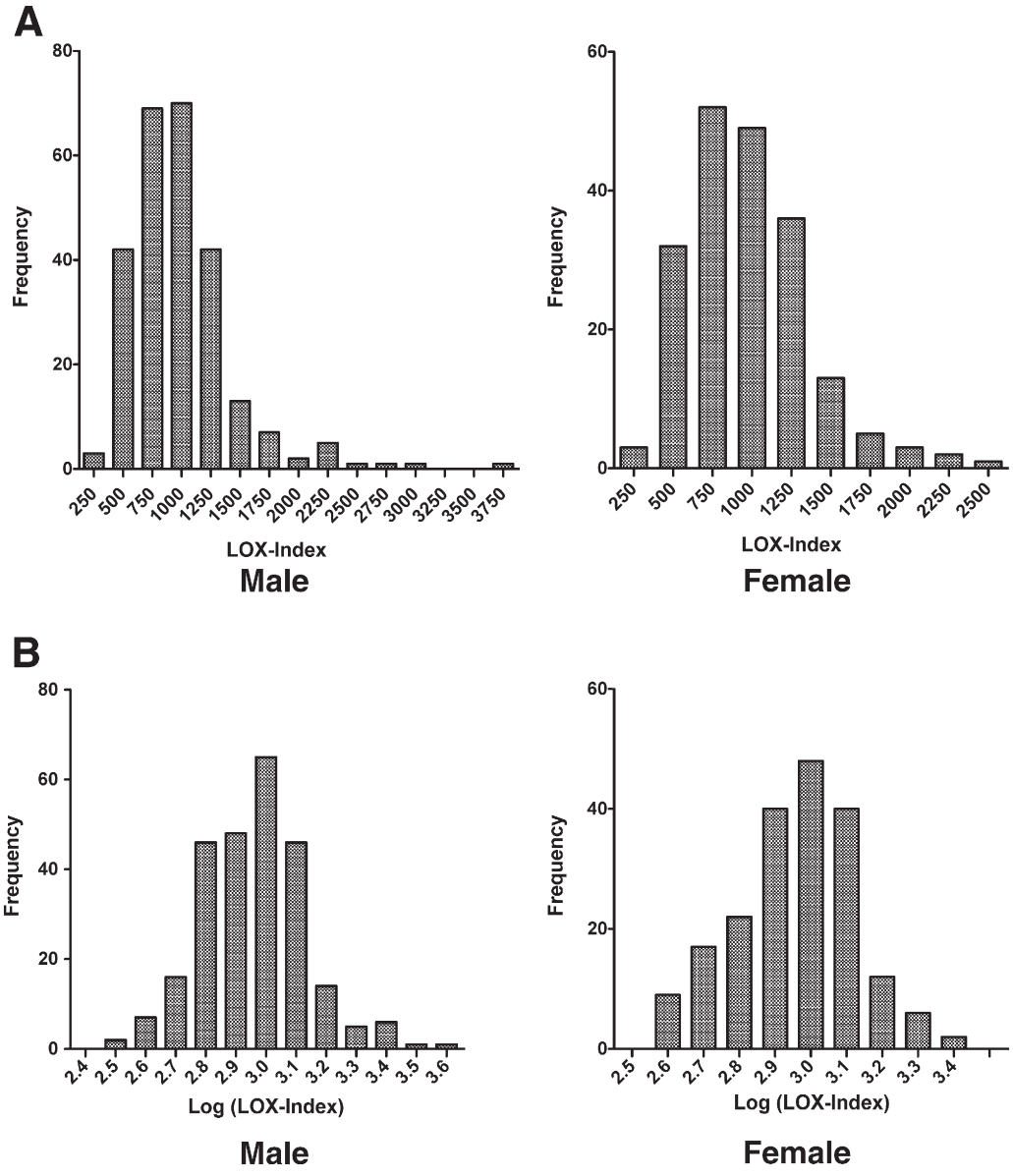

Fig. 1. A histogram for LOX-Index (A) and log-converted LOX-Index (B) in men and women. After log conversion, the LOX-index had normal distribution (Kolmogorov–Smirnov test: $p < 0.001$ for LOX-Index and $p = 0.200$ for log-converted LOX-Index).

3.2. Relation of LOX-Index and SDS

Among the enrolled subjects, 331 participants agreed to an evaluation of their mental status using SDS. There was no relationship between the log-converted LOX-Index and SDS scores as shown in Fig. 3.

4. Discussion

LOX-Index which is the product of LAB and sLOX-1, reflects the biological activity of LOX-1 ligands. It has been reported that the multivariable-adjusted hazard ratio for ischemic stroke and myocardial infarction from the second to top quartile of LOX-Index was three- and two-fold higher, respectively, than that for the bottom quartile after multivariable adjustment in a community-based cohort study, respectively [6]. On the other hand, FRS is a multivariable statistical model that uses age, sex, smoking history, BP, LDL-C, HDL-C, and blood glucose levels or history of diabetes to estimate coronary event risk among individuals without previously diagnosed coronary artery disease, and this risk stratification has been widely recommended [13]. In the present cross-sectional investigation, we observed that LOX-Index was significantly associated with the 10-year risk of FRS. This result strongly supports that LOX-Index is a predictor for CVD; therefore, LOX-Index is a comprehensive marker for the evaluation of the status of multiple risk factors.

The multiple linear regression analyses showed that the current smoking status, LDL, and HDL were associated with LAB. Recently, Wakabayashi et al. reported that the smoking status was one of the determinants of the LAB in healthy men [14]. Their findings were consistent with our results. Takanabe-Mori et al. also demonstrated that the sLOX-1 levels had a significantly positive relationship with the smoking-related parameters, such as the daily consumption of tobacco, or the log-transformed expired air carbon monoxide concentrations [15]. Therefore, the biological activity of LOX-1 was associated with the smoking status. In the present study, sLOX-1 was only weakly associated with the presence of diabetes. Previous investigations have demonstrated that sLOX-1 was associated with acute coronary syndrome [16]. It has been proposed that sLOX-1 was a biomarker for the acute phase of acute coronary syndrome. Furthermore, sLOX-1 was also strongly associated with inflammation. It is conceivable that the levels

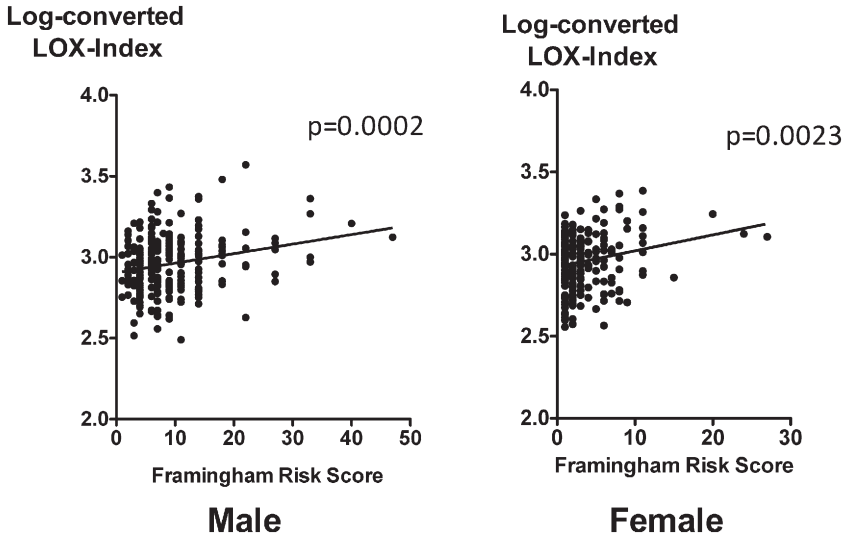

Fig. 2. The relationship between the 10-year risk of Framingham Risk Scores and log-converted LOX-Index. There was a significant positive correlation between the 10-year risk of FRS and log-converted LOX-Index in the male and female populations.

Table 2
Multiple regression analysis of log-converted LAB and sLOX-1.

	LAB (log-converted) ($R^2 = 14.5\%$)					sLOX-1 (log-converted) ($R^2 = 1.4\%$)				
	B	95%CI	β	p-Value	VIF	B	95%CI	β	p-Value	VIF
Age	0.001	0.000 to 0.002	0.062	0.197	1.213	0.000	−0.002 to 0.001	−0.021	0.666	1.213
Sex	−0.012	−0.033 to 0.009	−0.057	0.257	1.292	−0.005	−0.036 to 0.025	−0.019	0.729	1.292
LDL	0.001	0.001 to 0.001	0.249	<0.001*	1.109	0.000	0.000 to 0.001	0.025	0.619	1.109
HDL	−0.001	0.002 to 0.000	−0.165	0.001*	1.502	0.000	−0.001 to 0.001	0.048	0.403	1.502
TG(log)	−0.075	−0.120 to −0.030	−0.172	1.148	1.452	−0.004	0.071 to 0.063	−0.007	0.907	1.452
DM	0.002	−0.028 to 0.032	0.007	0.888	1.155	0.052	0.007 to 0.096	0.116	0.023*	1.155
Smoking	0.073	0.043 to 0.103	0.218	<0.001*	1.102	−0.010	−0.055 to 0.034	−0.023	0.646	1.102
HT	0.019	−0.005 to 0.042	0.078	0.114	1.244	0.000	−0.035 to 0.034	−0.001	0.989	1.244

B: coefficient, β: Standardized coefficient, VIF: variance inflation factor, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TG: triglyceride, DM: diabetes mellitus, HT: hypertension.
* p value of < 0.05 was considered statistically significant.

of sLOX-1 reflected acute stress or acute inflammation rather than chronic states. Since LAB reflected the state of dyslipidemia or smoking, LOX-Index could assess the cardiovascular risk from both side, that is, acute and chronic status.

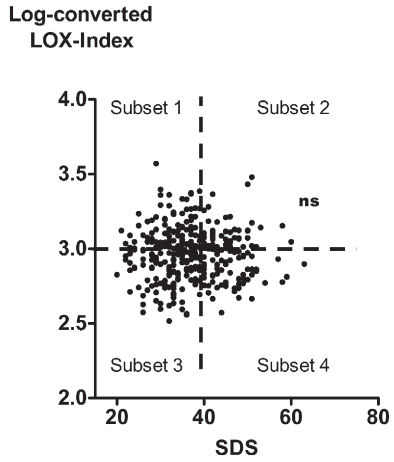

Fig. 3. The relationship between the SDS and the log-converted LOX-Index. There was no significant relationship between SDS and the log-converted LOX-Index. The calcification of health condition using the SDS and LOX-Index. It was based upon the presence or absence of cardiovascular risks and depression.

Previous investigations indicate a possibility that mental stress might be associated with oxidative stress. There was no significant association between LOX-Index and the SDS sores in the present investigation. However, a simultaneous evaluation of LOX-Index and SDS could be used to classify the subjects on the basis of two different aspects, oxidative and mental stress. The subjects with SDS scores over 40 points were defined as being depressed and those with the log-converted LOX-Index over 3.0 were defined as having cardiovascular risk due to oxidative stress according to the previous investigation. Therefore, all subjects were divided into four subsets as shown in Fig. 3. Subset 3 represented those with neither oxidative stress nor mental stress; subset 4 consisted of subjects with mental stress, but without oxidative stress; subset 1 consisted of subjects with oxidative stress, but without mental stress; subset 2 consisted of subjects with oxidative stress and mental stress. Thus, this classification system could contribute to the risk assessment for CVD and could help guide individual subjects.

Recent progress in pharmacotherapy has achieved a drastic decrease in the incidence of CVD. It has been established that statins can prevent the primary and secondary onsets of CVD. Further, as observed in a previous study, the combination of statins and eicosapentaenoic acids decreased the incidence of major coronary events by 19% in Japanese patients with a history of CAD. However, these powerful medications cannot completely eliminate CVD [17]. Therefore, significant residual cardiovascular risk remains even after the optimum treatment of

dyslipidemia. This residual risk depends upon several undetermined factors. Given the comprehensive significance, LOX-Index-guided therapeutic approach might lead to the resolution of the residual risk.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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