ORIGINAL RESEARCH

High Levels of Soluble Lectinlike Oxidized Low-Density Lipoprotein Receptor-1 Are Associated With Carotid Plaque Inflammation and Increased Risk of Ischemic Stroke

Hanna Markstad, MD;* Andreas Edsfeldt, MD, PhD;* Ingrid Yao Mattison, MSc; Eva Bengtsson, PhD; Pratibha Singh, PhD; Michele Cavalera, PhD; Giuseppe Asciutto, MD, PhD; Harry Björkbacka, PhD; Gunilla Nordin Fredrikson, PhD; Nuno Dias, MD, PhD; Petr Volkov, PhD; Marju Orho-Melander, PhD; Jan Nilsson, MD, PhD; Gunnar Engström, MD, PhD; Isabel Gonçalves, MD, PhD

***Background*—**When the lectinlike oxidized low-density lipoprotein (oxLDL) receptor-1 (LOX-1), a scavenger receptor for oxLDL, binds oxLDL, processes leading to endothelial dysfunction and inflammation are promoted. We aimed to study release mechanisms of LOX-1 and how circulating levels of soluble LOX-1 (sLOX-1) relate to plaque inflammation and future risk for ischemic stroke.

***Methods and Results*—**Endothelial cells and leukocytes were used to study release of sLOX-1. Plasma levels of sLOX-1 were determined in 4703 participants in the Malmö Diet and Cancer cohort. Incidence of ischemic stroke was monitored. For 202 patients undergoing carotid endarterectomy, levels of sLOX-1 were analyzed in plasma and plaque homogenates and related to plaque inflammation factors. Endothelial cells released sLOX-1 when exposed to oxLDL. A total of 257 subjects experienced stroke during a mean follow-up of 16.5 years. Subjects in the highest tertile of sLOX-1 had a stroke hazard ratio of 1.75 (95% CI, 1.28–2.39) compared with those in the lowest tertile after adjusting for age and sex. The patients undergoing carotid endarterectomy had a significant association between plasma sLOX-1 and the plaque content of sLOX-1 (r=0.209, P=0.004). Plaques with high levels of sLOX-1 had more oxLDL, proinflammatory cytokines, and matrix metalloproteinases.

***Conclusions*—**Our findings demonstrate that oxLDL induces the release of sLOX-1 from endothelial cells and that circulating levels of sLOX-1 correlate with carotid plaque inflammation and risk for ischemic stroke. These observations provide clinical support to experimental studies implicating LOX-1 in atherosclerosis and its possible role as target for cardiovascular intervention. **(*J Am Heart Assoc*. 2019;8:e009874. DOI: 10.1161/JAHA.118.009874.)**

Key Words: atherosclerosis • ischemic stroke • lectinlike oxidized low-density lipoprotein receptor-1 • soluble lectinlike oxidized low-density lipoprotein receptor-1

The role of lectinlike oxidized low-density lipoprotein (oxLDL) receptor-1 (LOX-1) in the development of atherosclerosis is receiving increasing attention.[1–3] LOX-1 is the main scavenger receptor for oxLDL on endothelial cells, but it is also expressed by macrophages, smooth muscle cells, and fibroblasts.[4,5] The expression of LOX-1 is low in resting cells and upregulated in response to proatherogenic factors, such as oxLDL, hyperglycemia, proinflammatory cytokines, and shear stress.[4,6,7] Binding of oxLDL to LOX-1 activates intracellular signal pathways, leading to increased expression of adhesion molecules, release of proinflammatory cytokines and matrix metalloproteinases (MMPs),[8,9] and stimulation of neoangiogenesis,[10] processes that play key roles in atherosclerotic plaque development and vulnerability.[11,12]

From the Center for Medical Imaging and Physiology, Skåne University Hospital, Lund University, Lund, Sweden (H.M.); Experimental Cardiovascular Research Unit, Clinical Research Center, Clinical Sciences Malmö, Lund University, Malmö, Sweden (H.M., A.E., I.Y.M., E.B., P.S., M.C., H.B., G.N.F., J.N., I.G.); Department of Cardiology (A.E., I.G.) and Vascular Center, Malmö, Sweden (G.A., N.D.), Skåne University Hospital, Malmö, Sweden; and Clinical Sciences Malmö, Lund University, Malmö, Sweden (P.V., M.O.-M., G.E.).

Accompanying Tables S1 through S4 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.009874

*Dr Markstad and Dr Edsfeldt contributed equally to this work.

Correspondence to: Hanna Markstad, MD, Thoracic Radiology, Center for Imaging and Physiology, Skåne University Hospital, Entrégatan 7, SE-22185 Lund, Sweden. E-mail: hanna.markstad@med.lu.se

Received May 22, 2018; accepted December 31, 2018.

Clinical Perspective

What Is New?

- The present study shows that plasma soluble lectinlike oxidized low-density lipoprotein receptor-1 is an independent predictor of future stroke and correlates with carotid plaque inflammation.

What Are the Clinical Implications?

- Soluble lectinlike oxidized low-density lipoprotein receptor-1 could herein be a potential clinical marker to identify patients at higher risk to experience a future stroke.

LOX-1 signaling has also been implicated in endothelial dysfunction by inhibition of NO synthase[13,14] and activation of apoptosis,[15] suggesting a possible role in cardiovascular events caused by endothelial erosions.[16,17] LOX-1 is abundantly expressed in atherosclerotic plaques,[18] and genetic single-nucleotide polymorphisms in the LOX-1 gene locus have been associated with increased risk for coronary artery disease.[1] Evidence directly linking LOX-1 to the development of atherosclerosis has come from studies demonstrating that LOX-1 gene deficiency reduces plaque development in LDL receptor knockout mice,[4] whereas overexpression aggravates atherosclerosis in apolipoprotein E–deficient mice.[19]

A soluble form of LOX-1 (sLOX-1) is present in the circulation.[20] sLOX-1 is released from cells after proteolytic cleavage of membrane-bound LOX-1,[21–23] and it has been assumed that sLOX-1 reflects the cellular expression of LOX-1.[24] Serum levels of sLOX-1 have been reported to be increased in patients with acute stroke,[25] but it has not been investigated if elevated levels of sLOX-1 are associated with an increased risk for future stroke. Herein, we analyzed baseline plasma levels of sLOX-1 in 4703 subjects from the Malmö Diet and Cancer (MDC) cohort and registered the incidence of ischemic stroke during a mean follow-up of 16.5 years. We also determined the association between plasma levels of sLOX-1 and carotid plaque inflammation in endarterectomy specimens from 202 patients taking part in the Carotid Plaque Imaging Project (CPIP).

Materials and Methods

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure. Because the material is human tissue and blood samples from biobank cohorts, it is limited. In addition, the local regulations and the ethical permission prohibit sample transfer.

MDC Cohort

During 1991 to 1996, all citizens between 45 and 73 years of age in the city of Malmö, the third largest city of Sweden, were invited to participate in the MDC study. A total of 28 449 individuals (60% women) completed the screening examinations (participation rate, 41%). A random sample of 6103 subjects was selected to participate in a further study of the epidemiological characteristics of carotid artery disease; of these, 5508 subjects donated fasting blood samples. The local ethical committee approved the study (LU 51–90). All patients gave informed consent.

After excluding subjects with previous stroke at baseline (n=48), those with missing baseline data (n=26), and analysis of plasma LDL or cholesterol (n=731), a total of 4703 samples were analyzed for sLOX-1 level.

Information on history of diabetes mellitus, the use of antidiabetic, blood pressure–lowering and lipid-lowering medication, and current smoking (regular or occasional basis) was obtained through a questionnaire at inclusion. Height, weight, and waist circumference were measured. Venous fasting blood samples were taken, cholesterol and glucose levels were analyzed on fresh samples, and LDL levels were calculated according to the Friedewald formula; EDTA plasma was frozen at −80°C immediately after centrifugation.

All participants were followed up from baseline examination until first hospitalization for acute ischemic stroke, death, emigration, or December 31, 2010, corresponding to a follow-up time of 16.5±3.6 years. Follow-up included hospital admission under the diagnosis cerebral infarction (code 434), according to the *International Classification of Diseases, Ninth Revision* (*ICD-9*). Death of participants was based on record linkage with the National Causes of Death Register. Sweden has a nationwide validated register in which >99% of all somatic and psychiatric hospital discharges and causes of death are registered.[26]

Thus, a total of 4703 subjects (2834 women and 1869 men) were available to study sLOX-1 in relation to incidence of ischemic stroke. Information on carotid plaque was available for 4530 of them. Complete information on all risk factors (age, sex, current smoking, diabetes mellitus, waist measurement, systolic blood pressure, LDL, and C-reactive protein [CRP]) was available for a total of 4594 subjects and for 4425 subjects with information about carotid plaque. Clinical characteristics of the MDC cohort are presented in Table S1.

Ultrasound of the Carotid Plaques in the MDC Cohort

Presence of carotid plaque in the right carotid artery was assessed through ultrasonography (Acuson XP4 System, Mountain View, CA) by trained certified ultrasonographers.

Scanning included 3 cm of the distal common carotid artery, the bifurcation, and 1 cm of the internal and external carotid artery. Plaque was defined as a focal thickening of the intima-media layer >1.2 mm and a minimum area of 10 mm^2.[27,28]

CPIP Cohort

A total of 202 patients, aged 69.5±8.4 years, who underwent carotid endarterectomy between November 2005 and October 2012 at our tertiary university hospital were included. From these 202 patients, 2 underwent bilateral carotid endarterectomy. All patients gave informed consent. The study was approved by the local ethical committee (472/2005). Clinical characteristics of the CPIP cohort are presented in Table S2.

Indications for surgery have been previously described[29] and are, in brief, the presence of symptoms (stroke, amaurosis fugax, or transient ischemic attack) and a degree of stenosis >70% or absence of symptoms and a degree of stenosis >80%. Blood samples were collected the day before surgery to measure sLOX-1. Circulating lipoproteins and CRP levels were analyzed on fresh samples in the local clinical laboratory.

All carotid plaques harvested by endarterectomy were flushed with saline to remove blood and debris before they were snap frozen in liquid nitrogen. Fragments (1-mm thick) from the most stenotic region of the plaque were removed for histological analysis. In 66 plaques, an extra fragment of similar size was collected for gene expression analyses. The rest of the plaque was homogenized, as described previously.[30]

Analyses of the Plaque Tissue: Cytokines and Chemokines

Interleukin-6, macrophage inflammatory protein-1β, soluble CD40 ligand, tumor necrosis factor-α (TNF-α), and fractalkine were assessed in plaque homogenate supernatants, according to the manufacturer's instruction (human cytokine/chemokine immunoassay; Millipore Corporation, MA), and analyzed with Luminex 100 IS 2.3 (Austin, TX).

Analyses of oxLDL and MMPs in Plaque Tissue

ELISA was used to measure oxLDL (ox-LDL; Mercordia, Uppsala, Sweden) in plaque homogenate supernatants, according to the manufacturer's instructions. MMPs 2 and 9 were analyzed in plaque homogenate supernatants using the Mesoscale human MMP ultrasensitive kit (Mesoscale, Gaithersburg, MD), according to the manufacturer's instructions.

Analyses of the Plaque Tissue: Histological and Immunohistochemistry Features

Cryosections (8 μm) of the most stenotic region of the plaque were fixed with Histochoice (Amresco, OH), dipped in 60% isopropanol and then in 0.4% Oil Red O in 60% isopropanol (for 20 minutes) to stain for neutral lipids. To assess the percentage of macrophages, a primary monoclonal mouse anti-human CD68 (DakoCytomation, Glostrup, Denmark; 1.85 μg/mL) and a secondary antibody polyclonal rabbit anti-mouse (DakoCytomation) were used. The percentage of vascular smooth muscle cell α-actin was assessed by using a primary antibody monoclonal mouse anti-human smooth muscle α-actin clone 1A4 (DakoCytomation; 0.71 μg/mL) and the secondary antibody biotin rabbit anti-mouse Ig (DakoCytomation). To assess the percentage of intraplaque hemorrhage, a primary antibody monoclonal mouse anti-human CD235a, against glycophorin A (M0819, clone JC159; DakoCytomation; 271 mg/L), and the secondary antibody biotinylated rabbit anti-mouse F(ab')2 (E0413; DakoCytomation; 0.79 g/L) were used. To assess the percentage of collagen I–positive area, a primary monoclonal mouse anti-human collagen I (Abcam, Ab6308) antibody and a secondary rabbit anti-mouse (Abcam, Ab98668) were used. Collagen III–positive areas were assessed using a primary monoclonal mouse anti-human collagen III (Abcam, Ab6310) and a secondary rabbit anti-mouse (Abcam, Ab98668). For the LOX-1 staining, the primary antibody mouse anti-human LOX-1 (Abcam 126538, Cambridge, England; 1 μg/mL) and relevant isotype control were used.

After incubation with an appropriate secondary antibody or MACH 3 Mouse HRP Polymer Detection kit (Biocare Medical), diaminobenzidine was used for detection and nuclei were counterstained with Mayer's hematoxylin.

Measurements of the percentage stained area of the plaque for the different stains were quantified blindly using BioPix iQ 2.1.8 (Gothenburg, Sweden) after scanning with ScanScope Console Version 8.2 (LRI Imaging AB, Vista, CA).

Analyses of the Plaque Tissue: oxLDL Receptor 1 Gene Expression Analysis

The gene expression of the LOX-1 regulating oxLDL receptor 1 gene was assessed in plaque tissue of 66 carotid plaques from global transcriptome RNA sequencing data. Libraries were sequenced on an Illumina HiSeq2000 platform, as previously described.[31]

Measurement of sLOX-1 in Blood Samples From Both Cohorts and Plaque Samples From CPIP

sLOX-1 was measured in EDTA plasma samples from both cohorts, MDC and CPIP, as well as in plaque homogenates

from CPIP, at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala University, using the Olink Proseek Multiplex kit. The samples from both cohorts had been stored frozen in −80°C. sLOX-1 was expressed as arbitrary units on a log2 scale. The within- and between-run coefficients of variation were 9% and 11%, respectively. Lower and upper limits of quantification correspond to 1.0 and 15 625 pg/mL, respectively (http://www.olink.com).

In Vitro Secretion of sLOX-1 by Stimulation of Human Peripheral Blood Mononuclear Cells, Coronary Artery Smooth Muscle Cells, and Umbilical Vein Endothelial Cells

Peripheral blood mononuclear cells (PBMCs) were isolated from a healthy blood donor using Ficoll-Paque Plus (GE Healthcare) and seeded at a density of 0.5×10^6 cells into a non–tissue-treated round-bottomed 96-well plate (Corning) in complete RPMI with 2% human serum (Sigma Aldrich). Human coronary artery smooth muscle cells (Thermo Fisher Scientific) and human umbilical vein endothelial cells (HUVECs; Lonza) were passaged and cultured in Medium 231 and Medium 200 (Thermo Fisher Scientific), respectively, including serum growth supplement to reach a confluency of ≈90%, continued by seeding into a tissue-cultured, flat-bottomed, 48-well plate (Corning) at a density of 0.2×10^5. Before stimulation, human coronary artery smooth muscle cells and HUVECs were serum starved in respective medium for 24 hours. PBMCs were stimulated with TNF-α (10–100 ng/mL), interleukin-1β (10 ng/mL), and transforming growth factor-β (1–10 ng/mL). PBMCs, human coronary artery smooth muscle cells, and HUVECs were also stimulated with copper-oxLDL (25 μg/mL), prepared as previously described by Niemann-Jönsson et al,[32] for 24 hours in 37°C with 5% CO_2. Other lipoprotein fractions were isolated, as previously described.[33,34] HUVECs were stimulated with native LDL (100 μg/mL), high-density lipoprotein (50 μg/mL), and very LDL (100 μg/mL), for 24 hours in 37°C with 5% CO_2.

To assess sLOX-1 in all cultures, medium was stored in −20°C and further analyzed using sLOX-1 human ELISA (Abcam), according to the company's instructions.

To assess cell viability, HUVECs were detached from the plate for 2 minutes in 0.25% trypsin (Sigma) diluted in PBS in room temperature. HUVECs and PBMCs were stained with Zombie aqua viability dye (Biolegend) and run immediately on a gallios flow cytometer (Beckman Coulter). Results were analyzed with FlowJo (Tree Star).

Statistical Analysis

Continuous variables were compared using the Student *t* test or the Mann-Whitney *U* test, depending on the variable distribution. Dichotomous variables were compared using the χ^2 test. Results were presented as mean±SD, median (interquartile range), or percentages, as appropriate. In vitro results are presented as mean±SEM or median and interquartile range. All statistical analyses were performed using IBM SPSS Statistics, version 24, or GraphPad Prism 7.

For MDC, the participants were divided into tertiles of sLOX-1, where tertile 1 represented the group with the lowest level of sLOX-1. Incidence of stroke was analyzed by sLOX-1 tertile. A Kaplan-Meier survival curve with the log-rank test was used to illustrate incidence of ischemic stroke per tertile of sLOX-1 (Figure 1). Cox proportional hazards regression was used to calculate hazard ratios in tertiles 2 and 3 of sLOX-1 compared with tertile 1 (reference) with corresponding CIs.

To further investigate the relation between incidence of stroke and presence of plaque, the participants were divided into 4 groups, depending on presence/absence of carotid plaque and high (tertile 3) versus moderate/low (tertile 1+tertile 2) levels of sLOX-1. Cox proportional regression was used with 2 different models adjusted for (1) age and sex; and (2) age, sex, current smoking, diabetes mellitus, waist measurement, systolic blood pressure, LDL, cholesterol, triglycerides, and CRP. The covariates were chosen on the basis of well-described clinical risk factors and potential confounding factors identified using a backward stepwise regression model with sLOX-1 as the dependent variable (Table S3).

For CPIP, all components measured in plaque homogenate were normalized to plaque wet weight, whereas the results of the histological and immunohistochemical analysis are presented as percentage of plaque area. Spearman's rank correlation or Mann-Whitney test was used because analyzed blood and plaque components were not normally distributed.

For the in vitro experiments, 1-way ANOVA or Kruskal-Wallis with additional Dunn's test were performed to assess sLOX-1 release from the cells.

Results

oxLDL and TNF-α Stimulate Release of sLOX-1

To investigate if oxLDL can induce shedding of sLOX-1, we exposed cultured HUVECs, human coronary artery smooth muscle cells, and PBMCs with 25 μg/mL of oxLDL for 24 hours. This resulted in an enhanced release of sLOX-1 from endothelial cells (Figure 2A), but not from smooth muscle cells or PBMCs (data not shown). No effect on sLOX-1 release from endothelial cells was detected when stimulating HUVECs with native LDL, high-density lipoprotein, or very LDL. Stimulation with interleukin-1β and transforming growth factor-β showed no significant release of sLOX-1. However, PBMCs could be stimulated to release sLOX-1 by exposure to

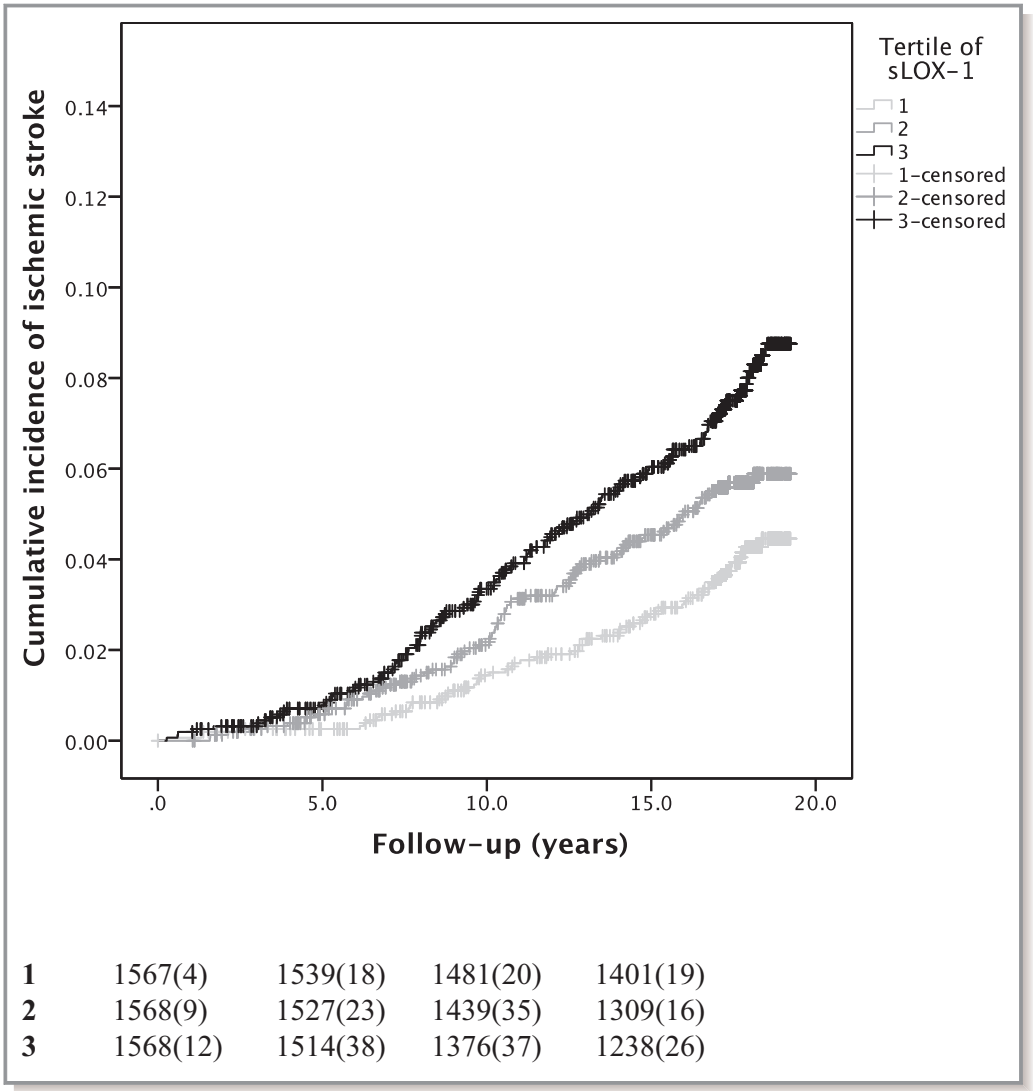

Figure 1. Kaplan-Meier survival curve per tertile of soluble lectinlike oxidized low-density lipoprotein receptor-1 (sLOX-1) in the Malmö Diet and Cancer cohort. The numbers below the figure denote the number of patients at risk per tertile of sLOX-1 and the number of events between parentheses. Log-rank test for trend across tertiles: $P<0.001$.

TNF-α (Figure 2B). The release of sLOX-1 from HUVECs was not associated with a significant reduction in cell viability. These observations show that circulating sLOX-1 may originate from cells exposed to oxLDL and other proinflammatory stimuli.

sLOX-1 and Ischemic Stroke

We next analyzed if there was any association between the baseline level of sLOX-1 in plasma and the risk for development of ischemic stroke in 4703 subjects participating in the MDC study. The clinical characteristics of the study cohort in relation to tertiles of sLOX-1 are presented in Table 1. The sLOX-1 levels increased with age and demonstrated significant associations with several cardiovascular risk factors, as well as with the presence of carotid plaques. Spearman correlations with sLOX-1 and CRP, lipids, and hemoglobin A1c are presented in Table S4. The magnitude of these correlations was moderate or low ($r\leq0.236$).

There was a significant association between the baseline plasma level of sLOX-1 and incidence of ischemic stroke during a mean follow-up of 16.5 years (Figure 1). Those in the highest tertile had a hazard ratio of 1.75 (95% CI, 1.28–2.39) compared with those in the lowest tertile after adjusting for age and sex. This association remained significant when further adjusting for current smoking, diabetes mellitus, waist circumference, systolic blood pressure, LDL, cholesterol, triglycerides, and CRP (Table 2). Because presence of carotid disease increases the risk for stroke, we next categorized

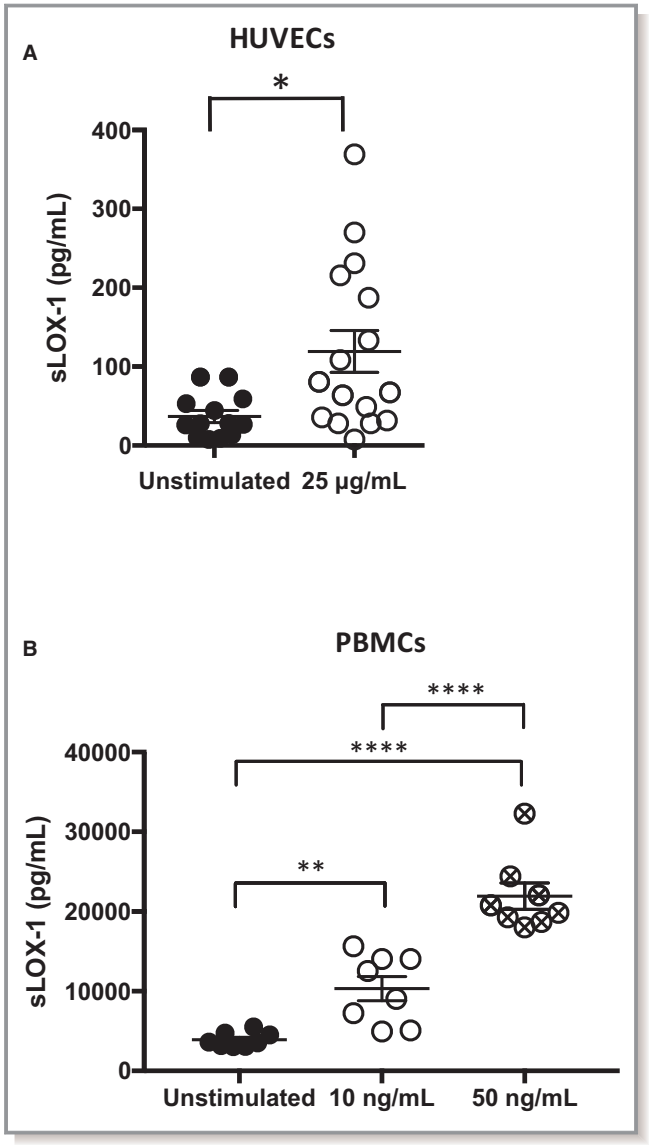

Figure 2. In vitro cultured human umbilical vein endothelial cells (HUVECs) stimulated with 25 µg/mL of oxidized low-density lipoprotein (oxLDL) induce a release of soluble lectinlike oxLDL receptor-1 (sLOX-1) into the medium (lines representing mean and SEM; results are pooled from 3 individual experiments) (**A**) and peripheral blood mononuclear cells (PBMCs) stimulated with tumor necrosis factor-α induce the release of sLOX-1 in a dose-dependent pattern (lines representing mean and SEM) (**B**). Each data point represents a single well replicate. Level of significance is marked: **P*=0.01, ***P*<0.01, *****P*<0.001.

subjects based on both sLOX-1 tertile and presence of carotid plaque. Subjects with a carotid plaque belonging to the highest tertile of sLOX-1 had a 2.4-fold higher risk than those without a plaque and belonging to the 2 lowest tertiles of sLOX-1 (*P*<0.001); and this difference remained significant when adjusting for age, sex, current smoking, diabetes mellitus, waist circumference, systolic blood pressure, LDL, cholesterol, triglycerides, and CRP (Table 3).

sLOX-1 and Plaque Inflammation

Experimental studies have shown that LOX-1 promotes plaque development and vulnerability through activation of vascular inflammation. To investigate the possible clinical relevance of this, we used plasma and plaques from 202 patients participating in the CPIP. Blood samples were obtained the day before endarterectomy. We used a 1-mm section from the most stenotic part of the removed plaque for histological and immunohistochemistry analyses, whereas homogenates of the remaining tissue were used for other analyses, including sLOX-1. There were highly significant associations between the plaque expression of proinflammatory cytokines/chemokines and sLOX-1 (sLOX-1 in plaque homogenates being the sum of sLOX-1 and cell-associated LOX-1, Table 4). Similar associations with plaque sLOX-1 levels were observed for MMP-2 and MMP-9, as well as for the percentage of lipid-stained area in histological sections of plaques (Table 4). The gene expression results were not significant for the LOX-1–regulating oxLDL receptor 1 gene. Interestingly, in plaque sections, LOX-1 staining primarily colocalized with staining for lipids and macrophages (Figure 3). There were also associations between the plaque content of the LOX-1 ligand oxLDL and the sLOX-1 in plaques (Table 4), as well as between levels of sLOX-1 in plaques and sLOX-1 in plasma (Figure 4). Plasma sLOX-1 correlated significantly with the plaque contents of oxLDL, TNF-α, fractalkine, MMP-9, and the percentage lipid-stained area in histological sections of plaques (Table 4).

Discussion

This study provides clinical support for a role of LOX-1 in cardiovascular disease by demonstrating that (1) exposure of endothelial cells to the LOX-1 ligand oxLDL increases the release of sLOX-1, (2) subjects with high circulating levels of sLOX-1 have more severe carotid disease and an increased risk of ischemic stroke, and (3) carotid plaques with high levels of sLOX-1 are characterized by increased inflammation. The cause of ischemic stroke is heterogeneous, but arterial atherothrombosis remains one of the most important causes.[35] Plaque rupture and endothelial erosion are the main triggers of acute atherothrombotic events.[36,37]

As LDL enters the vascular wall and becomes trapped in the extracellular matrix, it is oxidatively modified, becoming oxLDL. The oxLDL is taken up by inflammatory cells in an attempt to minimize the lesions and clear the excessive lipid accumulation. This process is suggested to be important for the initiation and progression of the disease, in some cases leading to the formation of vulnerable plaque, which eventually may rupture.

The processes preceding plaque rupture include extracellular accumulation of lipoprotein-derived lipids, death of

Table 1. Clinical Characteristics of the MDC Cohort According to Tertile of sLOX-1

Characteristics	sLOX-1 Tertile			*P* Value
	1 (n=1567)	2 (n=1568)	3 (n=1568)	
sLOX-1, mean±SD, au	3.48±0.279	4.04±0.134	4.79±0.480	<0.001
Age, mean±SD, y	56.6±5.97	57.9±5.95	58±5.88	<0.001
Male sex, n (%)	608 (38.8)	609 (38.8)	652 (41.6)	0.189
Current smoking, n (%)	186 (11.9)	324 (20.7)	499 (31.9)	<0.001
Diabetes mellitus, n (%)	90 (5.7)	107 (6.8)	159 (10.1)	<0.001
Use of antihypertensive medication, n (%)	223 (14.2)	240 (15.3)	288 (18.4)	0.005
Lipid-lowering treatment, n (%)	33 (2.1)	33 (2.1)	47 (3.0)	0.170
Waist, median (IQR), cm	81 (73–92)	82 (73–92)	83 (74–94)	<0.001
C-reactive protein, median (IQR), mg/L	1.00 (0.6–2.0)	1.30 (0.60–2.60)	1.80 (0.90–3.70)	<0.001
HbA1c, median (IQR), %	4.70 (4.40–5.00)	4.80 (4.50–5.10)	4.90 (4.60–5.20)	<0.001
Fasting lipoproteins, mmol/L				
Cholesterol, mean±SD	6.03±1.05	6.16±1.07	6.26±1.07	<0.001
Low-density lipoprotein, mean±SD	4.04±0.948	4.17±0.973	4.29±0.992	<0.001
High-density lipoprotein, median (IQR)	1.40 (1.17–1.66)	1.35 (1.12–1.61)	1.29 (1.08–1.54)	<0.001
Triglycerides, median (IQR)	1.05 (0.79–1.46)	1.16 (0.870–1.60)	1.23 (0.93–1.69)	<0.001
Atherosclerosis				
Presence of carotid plaque, %	28.7	34.3	38.8	<0.001
Stroke during follow-up, n (%)	61 (3.9)	83 (5.3)	113 (7.2)	<0.001

au indicates arbitrary units; HbA1c, hemoglobin A1c; IQR, interquartile range; MDC, Malmö Diet and Cancer; sLOX-1, soluble lectinlike oxidized low-density lipoprotein receptor-1.

reparative cells in the plaque, and degradation of fibrous tissue by MMPs, leading to formation of vulnerable plaques characterized by inflammation and a thin fibrous cap covering a necrotic core.[38] Rupture of vulnerable plaques is considered to cause approximately two thirds of all acute cardiovascular events.[37] The present observation that the carotid plaque content of sLOX-1 correlates to proinflammatory cytokines and MMPs in the plaque provides support for a role of LOX-1 in the formation of vulnerable atherosclerotic plaques. Enhanced degradation of the plaque fibrous tissue through the action of MMPs is a critical process in development of plaque vulnerability and rupture.[39] Genetic deletion of LOX-1 leads to reduced activation of MMP-2 and MMP-9 in hyperlipidemic animal models.[40] Exposure of cultured human endothelial cells to oxLDL results in increased MMP-9 expression, which is inhibited by adding LOX-1 blocking antibody.[41] Cardiovascular events not caused by plaque rupture are considered to be explained by endothelial erosions.[37] These are characterized by formation of a thrombus on top of a plaque with a thick fibrous cap without signs of rupture. The mechanisms responsible for development of endothelial erosions remain to be fully understood but are believed to involve endothelial dysfunction and death.[16] This makes LOX-1 a potential culprit in the

Table 2. Hazard Ratio for Future Ischemic Stroke per Tertile of sLOX-1

Model	sLOX-1 Tertile*			*P* Value	Per 1 SD*
	1	2	3		
1	1	1.27 (0.91–1.77)	1.75 (1.28–2.39)	<0.001	1.30 (1.16–1.46)
2	1	1.11 (0.79–1.56)	1.41 (1.01–1.96)	0.032	1.20 (1.06–1.14)

Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, current smoking, diabetes mellitus, waist, systolic blood pressure, low-density lipoprotein, cholesterol, triglycerides, and C-reactive protein. The *P* values are from the tests of trend across tertiles. sLOX-1 indicates soluble lectinlike oxidized low-density lipoprotein receptor-1.*Data are given as hazard ratio (95% CI).

Table 3. Hazard Ratio of Ischemic Stroke According to Presence or Absence of Carotid Plaque and High sLOX-1 (Tertile 3) or Low sLOX-1 (Tertile 1 or 2)

Variable	No Carotid Plaque		Carotid Plaque		*P* Value
	Low sLOX-1 (n=2078)	High sLOX-1 (n=916)	Low sLOX-1 (n=956)	High sLOX-1 (n=580)	
Ischemic stroke during follow-up, %	3.4	5.0	7.1	10.2	<0.001
Model 1*	1	1.45 (1.00–2.10)	1.64 (1.17–2.29)	2.42 (1.71–3.44)	<0.001
Model 2*	1	1.34 (0.92–1.96)	1.58 (1.12–2.24)	2.10 (1.45–3.04)	0.001

Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, current smoking, diabetes mellitus, waist, systolic blood pressure, low-density lipoprotein, cholesterol, triglycerides, and C-reactive protein. sLOX-1 indicates soluble lectinlike oxidized low-density lipoprotein receptor-1. *Data are given as hazard ratio (95% CI).

development of endothelial erosions in view of the experimental findings that activation of LOX-1 induces endothelial dysfunction and apoptotic signaling.[42] Accumulating evidence from observational clinical studies suggests that endothelial erosions are becoming an increasingly common cause of acute cardiovascular events, possibly as a result of a wider use of therapies targeting development of traditional plaque vulnerability.[43]

Our observations that subjects with high levels of sLOX-1 have more severe subclinical carotid disease also provide additional support for a role of LOX-1 in the development of atherosclerosis. Although this association does not per se prove a causal relationship, it is well in line with strong experimental evidence of a functional role of LOX-1 in atherosclerosis. Activation of endothelial LOX-1 results in expression of several factors known to be of key importance in atherosclerosis, including monocyte chemotactic protein-1, intracellular adhesion molecule-1, and vascular cell adhesion molecule-1, effects that can be blocked by treatment with LOX-1 antisense.[44,45] Genetic deletion of LOX-1 in LDL receptor–deficient mice reduced plaque development and inflammation without affecting the expression of NO synthase and anti-inflammatory cytokine interleukin-10.[13]

Despite accumulating evidence of a functional importance of LOX-1 in atherosclerosis and plaque vulnerability, the possible role of sLOX-1 as a biomarker of cardiovascular risk

Table 4. Spearman's Correlation Between the Plaque Levels of sLOX-1, the Plasma sLOX-1 Levels, and the Plaque Composition for Cytokines, Chemokines, Cell Markers, Lipids, Intraplaque Hemorrhage, MMPs, and Matrix Proteins in All Plaques (n=202)

Variable	Plaque sLOX-1		Plasma sLOX-1	
	r	*P* Value	*r*	*P* Value
Cytokines/chemokines, pg/g				
TNF-α	0.409	5×10^{-9}	0.214	3×10^{-3}
Interleukin-6	0.298	3×10^{-5}	0.099	0.17
sCD40L	0.286	7×10^{-5}	0.096	0.19
MIP-1β	0.230	1×10^{-3}	0.083	0.25
Fractalkine	0.323	6×10^{-6}	0.204	4×10^{-3}
Cell markers, % area				
α-Actin (smooth muscle cells)	−0.096	0.17	0.013	0.83
CD68 (macrophages)	0.115	0.10	0.037	0.54
Glycophorin A (hemorrhage)	0.176	1.3×10^{-2}	0.009	0.89
Plaque lipids				
Oil Red O, % area	0.226	1×10^{-3}	0.124	3×10^{-2}
OxLDL, μU/g	0.369	2×10^{-7}	0.216	3×10^{-3}
Plaque levels of MMP, pg/g				
MMP-2	0.322	5×10^{-6}	0.103	0.14
MMP-9	0.359	3×10^{-7}	0.151	3×10^{-2}

MIP indicates macrophage inflammatory protein; MMP, matrix metalloproteinase; oxLDL, oxidized low-density lipoprotein; sCD40L, soluble CD40 ligand; sLOX-1, soluble lectinlike oxLDL receptor-1; TNF, tumor necrosis factor; % area, percentage stained area of plaque.

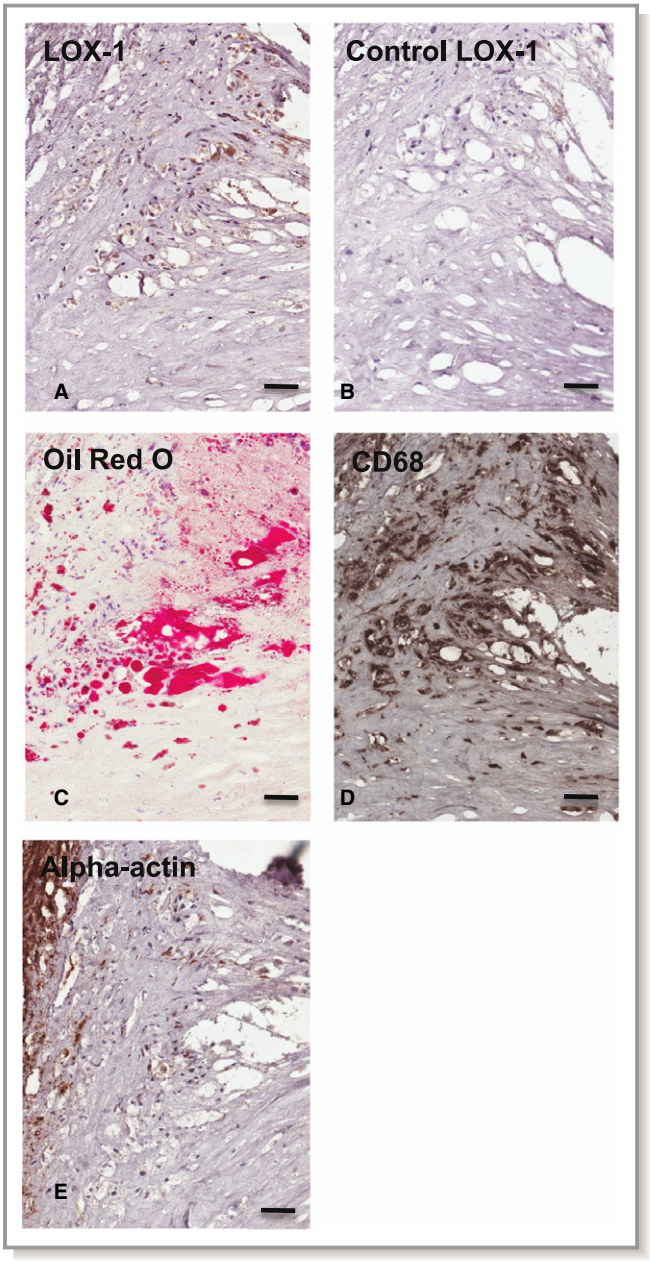

Figure 3. Immunohistochemistry showing the colocalization of lectinlike oxidized low-density lipoprotein receptor-1 (LOX-1), CD68, and neutral lipids (Oil Red O) in plaque tissue from the Carotid Plaque Imaging Project. **A**, LOX-1. **B**, Isotype control. **C**, Oil Red O (neutral lipids). **D**, CD68 (macrophages). **E**, α-Actin (smooth muscle cells). Bar=50 μm.

remains sparsely studied. Case-control studies have identified elevated levels of sLOX-1 in patients with stable and acute coronary disease, and these correlate with the severity of coronary disease.[46–49] Increased levels of sLOX-1 have also been identified in patients experiencing acute stroke.[25] In a prospective Japanese population study involving 2295 subjects with 60 ischemic strokes during an 11-year follow-up, Inoue and coworkers observed an association between a LOX-1/LOX-1 apolipoprotein B index and incident stroke but none with sLOX-1 alone.[50] Hence, this is the first large, prospective population study to show that high levels of sLOX-1 are associated with an increased risk for future ischemic stroke. The reasons for the different findings in these 2 studies are unclear but may involve differences in risk factor profiles between Japanese and Swedish populations. Notably, the risk associated with elevated sLOX-1 levels in our study was further increased by the presence of subclinical carotid disease. The reason for this remains to be fully clarified, but it could be speculated that LOX-1 becomes clinically more important in patients with established atherosclerosis. An important question is obviously if sLOX-1 level reflects membrane-bound LOX-1 signaling or if it has an inflammatory role itself. Our finding that endothelial cells exposed to oxLDL release sLOX-1 adds support to the former possibility but does not contradict the latter. However, it has also been shown by us and others that cytokines may induce the release of sLOX-1 from cells.[8] Our observations of significant associations between sLOX-1 and high-sensitivity CRP are in line with the possibility that elevated levels of sLOX-1 reflect an inflammatory state. On the other hand, it cannot be excluded that they also may reflect a role of sLOX-1 in inducing inflammation. Notably, we found strong associations between sLOX-1 and several cardiovascular risk factors, including age, smoking, diabetes mellitus, LDL, and low high-density lipoprotein. The biological mechanisms responsible for these associations also require further studies but may involve both increased formation of oxLDL and inflammation.

There are some limitations of the present study that need to be considered. Importantly, no causal relations can be extrapolated from observational studies such as the present. We found strong associations between sLOX-1 levels and several cardiovascular risk factors. It is possible that these risk factors activate the release of sLOX-1 but that this process is unrelated to the development of a cardiovascular event. However, the fact that the association between sLOX-1 and incident stroke remains significant when adjusting for these risk factors argues against this. Support for a functional role of LOX-1 in cardiovascular disease has come from mouse studies demonstrating that genetic deletion of LOX-1 reduces the development of atherosclerosis. The possible functional role of LOX-1 in human cardiovascular disease should be tested in mendelian randomization studies. Ultimately, randomized clinical trials targeting LOX-1 signaling need to be performed. In addition, the observation that sLOX-1 levels predict future strokes is only applicable on a presumably healthy population. Further studies determining if sLOX-1 can predict stroke also in subjects with established disease should be performed. It is likely that sLOX-1 levels vary over time, during the long-term follow up, and we cannot ensure that the individuals' sLOX-1

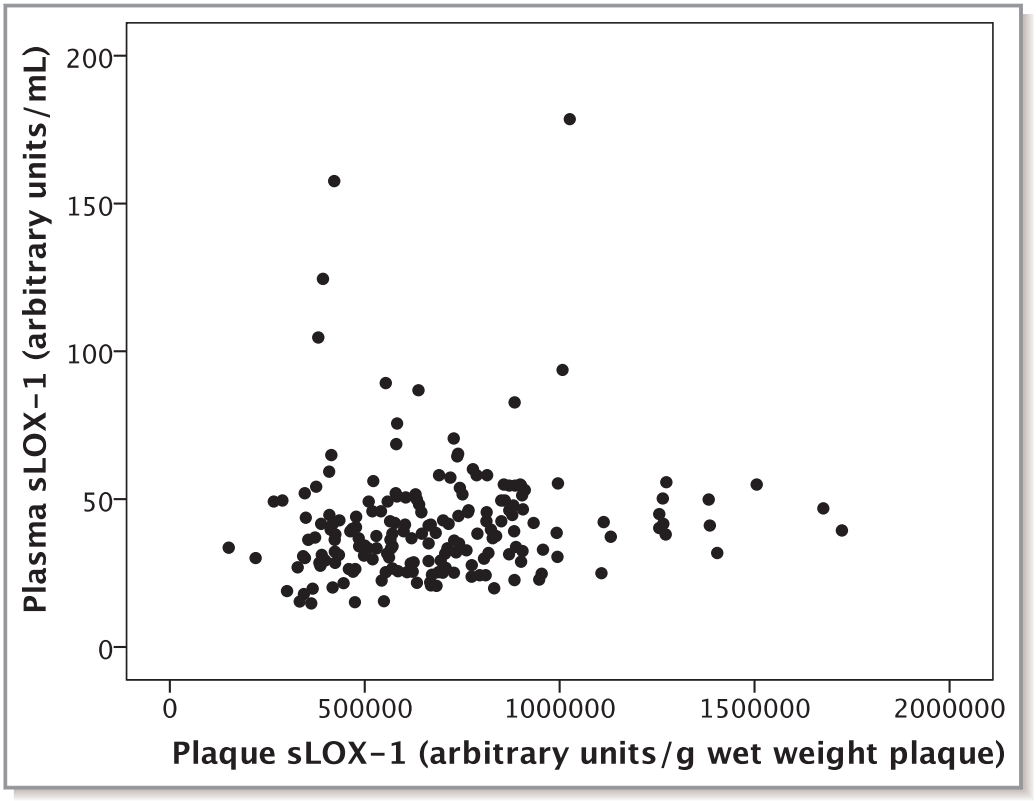

Figure 4. Scatter plot showing the positive correlation between plasma soluble lectinlike oxidized low-density lipoprotein receptor-1 (sLOX-1; arbitrary units/mL) and plaque sLOX-1 (arbitrary units/g wet weight plaque) content in the Carotid Plaque Imaging Project cohort (Spearman's correlation: r=0.209, P=0.004).

levels remained in the same tertile during the follow-up. The assay used to quantify sLOX-1 assesses all sLOX-1 cleaved at the 187 residue in the neck region. Therefore, we cannot be certain if it was primarily soluble (sLOX-1) or primarily cell-bound LOX-1 or even if tissue homogenization might have mechanically fragmented the cell-bound protein. However, this study has several interesting strengths: 2 large cohorts are evaluated, 1 of the cohorts has a long longitudinal follow-up and sLOX-1 is studied in both the circulation and human plaques.

In conclusion, the present study demonstrates that sLOX-1 is released from cells in response to oxLDL and proinflammatory cytokines and that the plasma level of sLOX-1 correlates with plaque inflammation and predicts risk for future stroke independently of major cardiovascular risk factors. In addition, the intraplaque content of sLOX-1 correlates strongly with the severity of plaque inflammation.

Besides antibodies and silencing approaches, there are several known modulators of LOX-1,[2] from naturally occurring plant compounds to medicines such as statins, calcium channel blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor type 1 blockers, and possibly even PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors, as the deletion of LOX-1 reduces PCSK9 in smooth muscle cells.[51] In a broader perspective, the present observations expand the understanding of LOX-1 as a possible future target for intervention.

Acknowledgments

We are grateful for excellent technical support from Ana Persson, Mihaela Nitulescu, and Lena Sundius; and writing and editing assistance from Mats Geijer.

Sources of Funding

This work was supported by the Swedish Research Council, Swedish Heart and Lung Foundation, Skåne University Hospital Foundations, Lund University Diabetes Center — Industrial Research Center from the Swedish Foundation of Strategic Research, Innovative Medicines Initiative (the SUMMIT consortium), Region Skåne Grants, Sparbank Foundation Färs & Frosta, Swedish Stroke Foundation, Swedish Society for Medical Research, Emil and Wera Cornell Foundation, Hjelt Foundation, and Diabetes Research and Wellness Foundation Sweden.

Disclosures

None.

References

1. Pothineni NVK, Karathanasis SK, Ding Z, Arulandu A, Varughese KI, Mehta JL. LOX-1 in atherosclerosis and myocardial ischemia: biology, genetics, and modulation. *J Am Coll Cardiol*. 2017;69:2759–2768.
2. Hofmann A, Brunssen C, Morawietz H. Contribution of lectin-like oxidized low-density lipoprotein receptor-1 and LOX-1 modulating compounds to vascular diseases. *Vascul Pharmacol*. 2017;107:1–11.
3. Arjuman A, Chandra NC. LOX-1: a potential target for therapy in atherosclerosis: an in vitro study. *Int J Biochem Cell Biol*. 2017;91:65–80.
4. Kume N, Murase T, Moriwaki H, Aoyama T, Sawamura T, Masaki T, Kita T. Inducible expression of lectin-like oxidized LDL receptor-1 in vascular endothelial cells. *Circ Res*. 1998;83:322–327.
5. Draude G, Hrboticky N, Lorenz RL. The expression of the lectin-like oxidized low-density lipoprotein receptor (LOX-1) on human vascular smooth muscle cells and monocytes and its down-regulation by lovastatin. *Biochem Pharmacol*. 1999;57:383–386.
6. Li L, Sawamura T, Renier G. Glucose enhances human macrophage LOX-1 expression: role for LOX-1 in glucose-induced macrophage foam cell formation. *Circ Res*. 2004;94:892–901.
7. Murase T, Kume N, Korenaga R, Ando J, Sawamura T, Masaki T, Kita T. Fluid shear stress transcriptionally induces lectin-like oxidized LDL receptor-1 in vascular endothelial cells. *Circ Res*. 1998;83:328–333.
8. Xu S, Ogura S, Chen J, Little PJ, Moss J, Liu P. LOX-1 in atherosclerosis: biological functions and pharmacological modifiers. *Cell Mol Life Sci*. 2013;70:2859–2872.
9. Li D, Liu L, Chen H, Sawamura T, Ranganathan S, Mehta JL. LOX-1 mediates oxidized low-density lipoprotein-induced expression of matrix metalloproteinases in human coronary artery endothelial cells. *Circulation*. 2003;107:612–617.
10. Wang X, Khaidakov M, Guo Z, Ding Z, He Q, Mehta JL. LOX-1 deletion limits cardiac angiogenesis in mice given angiotensin II. *Cardiovasc Drugs Ther*. 2014;28:441–446.
11. Hansson GK, Libby P, Tabas I. Inflammation and plaque vulnerability. *J Intern Med*. 2015;278:483–493.
12. Libby P, Lichtman AH, Hansson GK. Immune effector mechanisms implicated in atherosclerosis: from mice to humans. *Immunity*. 2013;38:1092–1104.
13. Mehta JL, Sanada N, Hu CP, Chen J, Dandapat A, Sugawara F, Satoh H, Inoue K, Kawase Y, Jishage K, Suzuki H, Takeya M, Schnackenberg L, Beger R, Hermonat PL, Thomas M, Sawamura T. Deletion of LOX-1 reduces atherogenesis in LDLR knockout mice fed high cholesterol diet. *Circ Res*. 2007;100:1634–1642.
14. Ryoo S, Bhunia A, Chang F, Shoukas A, Berkowitz DE, Romer LH. OxLDL-dependent activation of arginase II is dependent on the LOX-1 receptor and downstream RhoA signaling. *Atherosclerosis*. 2011;214:279–287.
15. Kataoka H, Kume N, Miyamoto S, Minami M, Morimoto M, Hayashida K, Hashimoto N, Kita T. Oxidized LDL modulates Bax/Bcl-2 through the lectinlike Ox-LDL receptor-1 in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 2001;21:955–960.
16. Quillard T, Franck G, Mawson T, Folco E, Libby P. Mechanisms of erosion of atherosclerotic plaques. *Curr Opin Lipidol*. 2017;28:434–441.
17. Nilsson J. Atherosclerotic plaque vulnerability in the statin era. *Eur Heart J*. 2017;38:1638–1644.
18. Kataoka H, Kume N, Miyamoto S, Minami M, Moriwaki H, Murase T, Sawamura T, Masaki T, Hashimoto N, Kita T. Expression of lectinlike oxidized low-density lipoprotein receptor-1 in human atherosclerotic lesions. *Circulation*. 1999;99:3110–3117.
19. Inoue K, Arai Y, Kurihara H, Kita T, Sawamura T. Overexpression of lectin-like oxidized low-density lipoprotein receptor-1 induces intramyocardial vasculopathy in apolipoprotein E-null mice. *Circ Res*. 2005;97:176–184.
20. Navarra T, Del Turco S, Berti S, Basta G. The lectin-like oxidized low-density lipoprotein receptor-1 and its soluble form: cardiovascular implications. *J Atheroscler Thromb*. 2010;17:317–331.
21. Murase T, Kume N, Kataoka H, Minami M, Sawamura T, Masaki T, Kita T. Identification of soluble forms of lectin-like oxidized LDL receptor-1. *Arterioscler Thromb Vasc Biol*. 2000;20:715–720.
22. Dreymueller D, Pruessmeyer J, Groth E, Ludwig A. The role of ADAM-mediated shedding in vascular biology. *Eur J Cell Biol*. 2012;91:472–485.
23. Mitsuoka H, Kume N, Hayashida K, Inui-Hayashiada A, Aramaki Y, Toyohara M, Jinnai T, Nishi E, Kita T. Interleukin 18 stimulates release of soluble lectin-like oxidized LDL receptor-1 (sLOX-1). *Atherosclerosis*. 2009;202:176–182.
24. Pirillo A, Catapano AL. Soluble lectin-like oxidized low density lipoprotein receptor-1 as a biochemical marker for atherosclerosis-related diseases. *Dis Markers*. 2013;35:413–418.
25. Yokota C, Sawamura T, Watanabe M, Kokubo Y, Fujita Y, Kakino A, Nakai M, Toyoda K, Miyamoto Y, Minematsu K. High levels of soluble lectin-like oxidized low-density lipoprotein receptor-1 in acute stroke: an age- and sex-matched cross-sectional study. *J Atheroscler Thromb*. 2016;23:1222–1226.
26. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, Heurgren M, Olausson PO. External review and validation of the Swedish national inpatient register. *BMC Public Health*. 2011;11:450.
27. Ostling G, Hedblad B, Berglund G, Goncalves I. Increased echolucency of carotid plaques in patients with type 2 diabetes. *Stroke*. 2007;38:2074–2078.
28. Rosvall M, Janzon L, Berglund G, Engstrom G, Hedblad B. Incidence of stroke is related to carotid IMT even in the absence of plaque. *Atherosclerosis*. 2005;179:325–331.
29. Asciutto G, Dias NV, Persson A, Nilsson J, Goncalves I. Treatment with betablockers is associated with higher grey-scale median in carotid plaques. *BMC Cardiovasc Disord*. 2014;14:111.
30. Goncalves I, Moses J, Dias N, Pedro LM, Fernandes e Fernandes J, Nilsson J, Ares MP. Changes related to age and cerebrovascular symptoms in the extracellular matrix of human carotid plaques. *Stroke*. 2003;34:616–622.
31. Edsfeldt A, Duner P, Stahlman M, Mollet IG, Asciutto G, Grufman H, Nitulescu M, Persson AF, Fisher RM, Melander O, Orho-Melander M, Boren J, Nilsson J, Goncalves I. Sphingolipids contribute to human atherosclerotic plaque inflammation. *Arterioscler Thromb Vasc Biol*. 2016;36:1132–1140.
32. Niemann-Jönsson A, Dimayuga P, Jovinge S, Calara F, Ares MP, Fredrikson GN, Nilsson J. Accumulation of LDL in rat arteries is associated with activation of tumor necrosis factor-alpha expression. *Arterioscler Thromb Vasc Biol*. 2000;20:2205–2211.
33. Dichtl W, Stiko A, Eriksson P, Goncalves I, Calara F, Banfi C, Ares MP, Hamsten A, Nilsson J. Oxidized LDL and lysophosphatidylcholine stimulate plasminogen activator inhibitor-1 expression in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 1999;19:3025–3032.
34. Dichtl W, Nilsson L, Goncalves I, Ares MP, Banfi C, Calara F, Hamsten A, Eriksson P, Nilsson J. Very low-density lipoprotein activates nuclear factor-kappaB in endothelial cells. *Circ Res*. 1999;84:1085–1094.
35. Ornello R, Degan D, Tiseo C, Di Carmine C, Perciballi L, Pistoia F, Carolei A, Sacco S. Distribution and temporal trends from 1993 to 2015 of ischemic stroke subtypes: a systematic review and meta-analysis. *Stroke*. 2018;49:814–819.
36. Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. *N Engl J Med*. 2013;368:2004–2013.
37. Falk E, Nakano M, Bentzon JF, Finn AV, Virmani R. Update on acute coronary syndromes: the pathologists' view. *Eur Heart J*. 2013;34:719–728.
38. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. *Circ Res*. 2014;114:1852–1866.
39. Newby AC. Metalloproteinases and vulnerable atherosclerotic plaques. *Trends Cardiovasc Med*. 2007;17:253–258.
40. Hu C, Dandapat A, Sun L, Chen J, Marwali MR, Romeo F, Sawamura T, Mehta JL. LOX-1 deletion decreases collagen accumulation in atherosclerotic plaque in low-density lipoprotein receptor knockout mice fed a high-cholesterol diet. *Cardiovasc Res*. 2008;79:287–293.
41. Li L, Renier G. The oral anti-diabetic agent, gliclazide, inhibits oxidized LDL-mediated LOX-1 expression, metalloproteinase-9 secretion and apoptosis in human aortic endothelial cells. *Atherosclerosis*. 2009;204:40–46.
42. Hong D, Bai YP, Gao HC, Wang X, Li LF, Zhang GG, Hu CP. Ox-LDL induces endothelial cell apoptosis via the LOX-1-dependent endoplasmic reticulum stress pathway. *Atherosclerosis*. 2014;235:310–317.
43. Libby P, Pasterkamp G. Requiem for the "vulnerable plaque." *Eur Heart J*. 2015;36:2984–2987.
44. Li D, Mehta JL. Antisense to LOX-1 inhibits oxidized LDL-mediated upregulation of monocyte chemoattractant protein-1 and monocyte adhesion to human coronary artery endothelial cells. *Circulation*. 2000;101:2889–2895.
45. Li D, Chen H, Romeo F, Sawamura T, Saldeen T, Mehta JL. Statins modulate oxidized low-density lipoprotein-mediated adhesion molecule expression in human coronary artery endothelial cells: role of LOX-1. *J Pharmacol Exp Ther*. 2002;302:601–605.
46. Hayashida K, Kume N, Murase T, Minami M, Nakagawa D, Inada T, Tanaka M, Ueda A, Kominami G, Kambara H, Kimura T, Kita T. Serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are elevated in acute coronary syndrome: a novel marker for early diagnosis. *Circulation*. 2005;112:812–818.
47. Balin M, Celik A, Kobat MA. Circulating soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are associated with proximal/middle segment of

the LAD lesions in patients with stable coronary artery disease. *Clin Res Cardiol*. 2012;101:247–253.

48. Kobayashi N, Takano M, Hata N, Kume N, Yamamoto M, Yokoyama S, Shinada T, Tomita K, Shirakabe A, Otsuka T, Seino Y, Mizuno K. Soluble lectin-like oxidized LDL receptor-1 (sLOX-1) as a valuable diagnostic marker for rupture of thin-cap fibroatheroma: verification by optical coherence tomography. *Int J Cardiol*. 2013;168:3217–3223.
49. Zhao ZW, Zhu XL, Luo YK, Lin CG, Chen LL. Circulating soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are associated with angiographic coronary lesion complexity in patients with coronary artery disease. *Clin Cardiol*. 2011;34:172–177.
50. Inoue N, Okamura T, Kokubo Y, Fujita Y, Sato Y, Nakanishi M, Yanagida K, Kakino A, Iwamoto S, Watanabe M, Ogura S, Otsui K, Matsuda H, Uchida K, Yoshimoto R, Sawamura T. LOX index, a novel predictive biochemical marker for coronary heart disease and stroke. *Clin Chem*. 2010;56:550–558.
51. Ding Z, Liu S, Wang X, Deng X, Fan Y, Shahanawaz J, Shmookler Reis RJ, Varughese KI, Sawamura T, Mehta JL. Cross-talk between LOX-1 and PCSK9 in vascular tissues. *Cardiovasc Res*. 2015;107:556–567.

SUPPLEMENTAL MATERIAL

Table S1. Clinical characteristics of the Malmö Diet and Cancer cohort, mean follow-up time 16.5 years.

sLOX-1, mean ±SD	4.1 ±0.63
Age (years), mean ±SD	57 ±6.0
Males, n (%)	1869 (40)
Current smoking, n (%)	1009 (22)
Diabetes, n (%)	356 (7.6)
Incidental stroke by last follow-up, n (%)	257 (5.5)
Carotid plaque, n (%)	1536 (33)
Use of anti-hypertensive medication, n (%)	751 (16)
Lipid-lowering treatment, n (%)	113 (2.4)
Waist (cm), median (IQR)	82 (73-92)
C-reactive protein (mg(L), median (IQR)	1.3 (0.7-2.8)
HbA1c %, median (IQR)	4.8 (4.5-5.1)
Fasting lipoproteins (mmol/L)	
Cholesterol, mean ±SD	6.2 ±1.1
Low-density lipoprotein, mean ±SD	4.2 ±0.98
High-density lipoprotein, median (IQR)	1.3 (1.1-1.6)
Triglycerides, median (IQR)	1.2 (0.86-1.6)

Table S2. Clinical characteristics of the cohort (n=202) who underwent carotid endarterectomy, donating plaque and blood samples to the Carotid Plaque Imaging Project (CPIP) part of the study.

Age years, median (IQR)	70 (64-75)
Males, n (%)	138 (68)
Smoking, n (%)	
current smokers	67 (33)
past smokers	95 (47)
non-smokers *	40 (20)
Diabetes, n (%)	69 (34)
Hypertension,† n (%)	151 (75)
Lipid-lowering treatment, n (%)	177 (88)
Degree of stenosis %, median (IQR)	90 (80-95)
Preoperative symptoms, n (%)	107 (53)
C-reactive protein (mg/L), median (IQR)	3.9 (1.9-6.5)
HbA1c (mmol/mol), median (IQR)	51 (45-64)
Fasting lipoproteins (mmol/L), median (IQR)	
Cholesterol	4.3 (3.5-5.1)
Low-density lipoprotein	2.4 (1.9-3.1)
High-density lipoprotein	1.1 (0.9-1.3)
Triglycerides	1.3 (1-1.8)

* not active smoking for at least 6 months before surgery; † systolic blood pressure > 140 mmHg or anti-hypertensive treatment.

Table S3. Age and sex in addition to all parameters significantly correlated with sLOX-1 levels in a backward stepwise multiple variable linear regression model.

	Full model (First step)		After backward elimination (Last step)	
	Beta coefficient*	*p*-value	Beta coefficient*	*p*-value
Age	0.058	<0.001	0.058	<0.001
Males	-0.006	0.803	-	-
Current smoking (yes vs. no)	0.295	<0.001	0.296	<0.001
Diabetes (yes vs. no)	0.018	0.670	-	-
Use of anti-hypertensive medication (yes vs. no)	0.039	0.137	-	-
Use of blood-lipid lowering medication (yes vs. no)	0.039	0.522	-	-
Waist	-0.027	0.207	-	-
C-reactive protein	0.125	<0.001	0.124	<0.001
HbA1c	0.045	0.011	0.048	0.001
Cholesterol	-0.141	0.547	-0.124	0.008
Low-density lipoprotein	0.202	0.347	0.185	<0.001
High-density lipoprotein	-0.001	0.989	-	-
Triglycerides	0.100	0.124	0.095	<0.001
Presence of carotid plaque (yes vs. no)	0.031	0.120	0.034	0.082

*Beta coefficient in relation to one standard deviation increment for continuous variables or unstandardized beta coefficient for dichotomous variables.

Table S4. Spearman correlation between sLOX-1 and C-reactive protein as a marker of inflammation, HbA1c and circulating lipoproteins.

sLOX-1	r	*p*-value
C-reactive protein	0.236	<0.001
HbA1c	0.157	<0.001
Cholesterol	0.096	<0.001
Low-density lipoprotein	0.108	<0.001
High-density lipoprotein	-0.122	<0.001
Triglycerides	0.160	<0.001