

Title

Weight reduction can decrease circulating soluble lectin-like oxidized LDL receptor-1 (sLOX-1)

levels in over-weight middle-aged men

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Acknowledgements

This work was supported in part by the Foundation for Total Health Promotion (2006), the Tanaka Project (2004-2006) of the Tsukuba Advanced Research Alliance (TARA) Center at the University of Tsukuba, a grant-in-aid from the Japanese Ministry of Health, Labour and Welfare (2005-2007, Kita Project), and the 21st century Center of Excellence (COE) program, Ministry of Education, Culture, Sports, Science and Technology (2002-2006, Nishihira Project).

Running title

sLOX-1 decreased with weight reduction

Abstract

Circulating soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) has been reported to be associated with acute coronary syndrome (ACS), but its association with obesity has not been elucidated. In this study, we examined whether weight reduction would reduce the serum levels of sLOX-1 in a 12-week weight-reduction intervention. Thirty-eight over-weight middle-aged men were enrolled in the study and 32 completed the intervention. The serum level of sLOX-1 was measured using a chemiluminescent enzyme-linked immunoassay. Following the intervention program, body weight and the serum level of sLOX-1 decreased significantly ($-7.5\% \pm 4.8\%$ and $-72.1\% \pm 35.9\%$, respectively). Changes in serum levels of sLOX-1 were positively correlated with changes in body weight ($r = 0.54$, $P = 0.003$), body mass index (BMI) ($r = 0.57$, $P = 0.001$), body fat mass ($r = 0.57$, $P = 0.002$), total cholesterol ($r = 0.41$, $P = 0.03$), subcutaneous fat area ($r = 0.50$, $P = 0.007$), high sensitive C-reactive protein (hsCRP) ($r = 0.56$, $P = 0.002$), leptin ($r = 0.47$, $P = 0.01$)

and tumor necrosis factor- α ($r = 0.32$, $P = 0.09$), but no correlations were observed with fasting glycemic related factors (blood glucose, hemoglobin A1c, insulin). Changes in BMI and hsCRP were selected as significant predictors of sLOX-1 changes by multiple regression analyses. These results suggest that LOX-1 induction may be related to adipocyte metabolism, inflammation, and immune response associated with obesity.

Key words: soluble lectin-like oxidized LDL receptor-1, obesity, weight reduction, inflammation,

adipocytokine

1. Introduction

Acute coronary syndrome (ACS) is one of the major causes of mortality and morbidity in developed countries. Since accurate diagnosis of ACS at the earliest stage can improve the prognosis of patients, a sensitive and specific early biomarker for ACS would be desirable. Moreover, oxidized low-density lipoprotein (Ox-LDL) and its receptor appear to play key roles in atherogenesis and the process of atherosclerotic plaque destabilization, erosion, and rupture, which are the major causes of ACS [1,2].

Lectin-like oxidized LDL receptor-1 (LOX-1), a type II membrane glycoprotein acting as a receptor for Ox-LDL, mediates Ox-LDL induced vascular dysfunction [3–6]. LOX-1 is cleaved from the cell surface by certain protease activities, released as soluble LOX-1 (sLOX-1). Circulating sLOX-1 levels are elevated in individuals with ACS, reflecting its prominent expression and enhanced protease activities in vulnerable atherosclerotic plaques in situ, and those circulating

LOX-1 levels can be a useful biomarker for ACS [7,8]. ACS is also thought to involve proinflammatory and immune responses [9,10]. Lubrano V et al. [11] have reported sLOX-1 being associated with inflammatory markers. Obesity, on the other hand, can be characterized by a state of chronic low-grade inflammation [12,13]. Long-term activation of proinflammatory pathways may be a mechanism for the development of insulin resistance [14], while serum concentrations of various cytokines are increased in obese individuals and may decrease after weight reduction [15-17].

However, the pathophysiological roles of sLOX-1 remain unclear. That is, the relationships between circulating sLOX-1 levels and inflammatory factors in obese individuals and the effect of weight reduction on circulating sLOX-1 levels are still unknown. Therefore, the aims of this study were 1) to assess whether weight reduction affects expression of sLOX-1 and 2) to assess whether changes in serum levels of sLOX-1 vary among individuals and whether they are associated with changes in anthropometric and metabolic parameters, adipocytokines, proinflammatory cytokines,

and high sensitive C-reactive protein (hsCRP) brought about by weight reduction in over-weight middle-aged men.

2. Methods

2.1. Subjects

Thirty-eight Japanese men were recruited through advertisements in a local newspaper.

Participants were excluded if they had a body mass index (BMI) less than 25 kg/m²; were smokers;

5 had concomitant renal, hepatic or cardiac disease; and/or were being treated with drugs that could

affect the variables of the study. Six subjects were unable to complete the study successfully for

personal reasons. This left 32 men, aged 32-66 years, to be measured. Assays and measurements

were carried out at 7days before onset of the intervention and 10days after a 12-week

weight-reduction intervention. The aim and design of the study were explained to all subjects before

10 they gave their written consent. In addition, the study conformed to the principles outlined in the

Helsinki Declaration and was approved by the Review Board of the University of Tsukuba.

2.2. Dietary and exercise protocol

All subjects were instructed to have a well-balanced 1680 kcal meal per day during the 12-week intervention. Subjects kept daily food diaries during this period and received weekly lectures and counseling from skilled dietitians. In addition to diet, subjects performed an exercise program that

5 consisted of 36 walking and jogging sessions (three days per week), which were supervised by two or three exercise instructors. In the first two months, the exercise program entailed only walking, with the target Borg's scale [18] ranging from 11 (light) to 13 (fairly hard). In the last month, subjects performed a combination of a 3.0 km of brisk walking and 1.0 km of middle-intensity jogging, with the target Borg's scale ranging from 13 (fairly hard) to 15 (hard). Subjects measured

10 their heart rates by palpation while walking or jogging, and recorded the duration (minutes) and intensity (heart rate or the Borg's scale) of each exercise session.

2.3. Clinical variables

Body weight was measured to the nearest 0.1 kg using a digital scale. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, while body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). A dual-energy X-ray absorptiometry (DXA) machine (DPX-NT, Lunar, Madison, Wisconsin, USA) was used to evaluate body composition, which consisted of fat tissue and lean soft tissue. Visceral fat area (VFA) and subcutaneous fat area (SFA) were measured at the level of the umbilicus (L4-L5) using computed tomography (CT) scans (Somatom AR.C, Siemens, Erlangen, Germany), which were performed on subjects in the supine position. VFA and SFA were calculated using a computer software program (FatScan, N2system, Osaka, Japan). Systolic and diastolic blood pressure (SBP and DBP) were measured with a sphygmomanometer, while maximal oxygen uptake (VO_2max) was determined during a graded exercise test using a cycle ergometer (828E, Monark, Stockholm, Sweden). Following a two-minute

warm-up, subjects started with a workload of 30 watts, which was increased by 15 watts each minute until volitional exhaustion occurred. Pulmonary ventilation and gas exchange were measured breath-by-breath with an on-line data acquisition system (Oxycon alpha System, Mijnhardt, Breda, Netherlands).

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2.4. Blood analyses

A blood sample was drawn from each subject after a 12-hour fast. Serum glucose and lipids were assayed by routine automated laboratory methods while hemoglobin A1c was measured by ion-exchange high performance liquid chromatography (HPLC) methods (Bio-Rad Inc, California, USA). Insulin was measured by an enzyme immunoassay method (Tosoh, Tokyo, Japan), and high sensitive CRP was measured by particle-enhanced immunoturbidimetric assay (Roche Diagnostics, GmbH, Mannheim, Germany). Concentrations of serum LOX-1 were measured by a sandwich

chemiluminescent ELISA using two different human LOX-1-specific monoclonal antibodies with a recombinant human LOX-1 extracellular domain as an assay standard, which was modified from the previously described sandwich ELISA [19]. Monoclonal antibodies directed to human LOX-1 were established by standard hybridoma techniques after immunizing mice with a recombinant protein corresponding to the extracellular domain of human LOX-1. The serum leptin, tumor necrosis factor (TNF)- α , and interleukin (IL)-6 levels were measured with an ELISA kit (R&D systems, Minneapolis, USA). The serum adiponectin levels were measured with an ELISA kit (Otsuka pharmaceutical Co, Ltd, Tokyo, Japan). Intra- and inter-assay coefficients of variation (CVs) were 4.1% and 7.6% for LOX-1, 2.8% and 3.2% for leptin, 11.8% and 6.2% for TNF- α , and 7.3% and 3.5% for IL-6, 2.2% and 1.8% for adiponectin, respectively (n = 32).

2.5. Statistical analysis

Data were presented as mean \pm standard deviation, and paired t tests were used to assess differences between variables before and after the weight reduction program. Relationships between two measurement variables were assessed by Pearson's product moment correlation, while stepwise multiple regression analyses were used to estimate the independent contribution of the selected variables to the change in sLOX-1 concentration in response to weight reduction. A value of $P < 0.05$ was considered to be statistically significant. All analyses were performed using SPSS software version 11.5 J for Windows (SPSS, Chicago, IL).

3. Results

Attendance at this intervention (36 sessions) averaged 80% (range 50%–100%) for the subjects.

The frequency of the exercise program was 2.4 ± 0.6 days/week with an average duration of 95 ± 27 min/week.

5 As shown in Table 1, body weight, BMI, fat mass, % fat mass, serum lipid, and visceral fat decreased significantly and maximal oxygen uptake ($VO_2\text{max}$) increased significantly. With the change in weight, serum sLOX-1 level decreased $72.1\% \pm 35.9\%$. There were only two subjects with slight increases in serum sLOX-1 level ($\Delta 1.1\text{pg/ml}$, $\Delta 6.6\text{ pg/ml}$) while showing decreases in weight ($\Delta -10.3\%$, $\Delta -5.4\%$), fat mass ($\Delta -3.8\%$, $\Delta -11.0\%$), and visceral fat area ($\Delta -43.8\%$, $\Delta -30.4\%$) (Fig
10 1A, B).

As shown in Table 2, baseline serum sLOX-1 was positively correlated with baseline body weight ($r = 0.473$, $P = 0.01$) and BMI ($r = 0.453$, $P = 0.02$). Changes in sLOX-1 correlated positively

with changes in body weight ($r = 0.542$, $P = 0.003$), BMI ($r = 0.574$, $P = 0.001$), and fat mass ($r = 0.570$, $P = 0.002$). No significant correlations were seen with fasting glycemc related factors (blood glucose, hemoglobin A1c, insulin) and VO_2 max at baseline or with changes. Correlations changes sLOX-1 with changes BMI ($r = 0.437$, $P = 0.023$) and fat mass ($r = 0.462$, $P = 0.015$) were significant even after adjustment for body weight.

High sensitive CRP, TNF- α , IL-6, leptin, and adiponectin decreased significantly (Table 1).

Baseline sLOX-1 was positively correlated with baseline hsCRP ($r = 0.58$, $P = 0.001$), while changes in sLOX-1 correlated positively with hsCRP ($r = 0.56$, $P = 0.002$). These associations remained significant after adjustment body weight. Changes in sLOX-1 correlated positively with leptin ($r = 0.47$, $P = 0.01$), and TNF- α ($r = 0.32$, $P = 0.09$) (Table 2). Stepwise multiple regression analysis indicated that changes in BMI and hsCRP were significant predictors of change in sLOX-1 ($R^2 = 0.459$), as shown in Table 3.

4. Discussion

Proteolytic cleavage of LOX-1 releases a soluble form of the receptor. Since the level of soluble receptors in circulating blood may reflect expression of membrane proteins and disease activities, sLOX-1 may be a potential biomarker of vascular disease and acute coronary syndrome [20,21].

5 Brinkly et al. [22] have reported that LOX-1 polymorphisms may be associated with plasma sLOX-1 levels.

In the current study, the following three findings were obtained. First, serum sLOX-1 levels decreased significantly with weight reduction. Second, basal serum sLOX-1 levels correlated positively with basal body weight and BMI. Changes in serum sLOX levels correlated positively
10 with changes in body weight, BMI, fat mass, abdominal fat area, and serum lipid, but no significant correlations were observed with glycemic related factors and VO₂max. Third, changes in sLOX-1 levels were positively correlated with changes in hsCRP and leptin. They were also marginally

correlated with TNF- α , whereas no correlations were observed with IL-6. Changes in BMI and hsCRP were significant predictors for change in sLOX-1 concentration.

According to our observations, weight reduction contributed not only to lowering circulating sLOX-1 levels but also to reducing fat mass, abdominal fat area, and serum lipids, all of which are well-known risk factors for vascular disease. Changes in sLOX-1 levels were also significantly correlated with changes in the factors mentioned above. These results suggest that LOX-1 expression may be related to adipocyte metabolism. Brinkley et al. [23] reported that plasma levels of sLOX-1 were significantly correlated with body weight, BMI, and total body fat. While their study may be the first to report an interaction between serum sLOX-1 levels and obesity, it supports our data here.

Obesity is also associated with alterations in immune function, but the effect of obesity itself on immune system function is variable [24]. Obese subjects seem to primarily have impairment in immune response that is reversible with weight reduction [14,15]. Serum sLOX-1 level increased

because LOX-1 can be induced by proinflammatory stimuli [11,25]. Indeed, Honjo et al. [26] found that LOX-1 is involved in endotoxin-induced inflammation as well as leukocyte recruitment and infiltration in vivo, and these studies suggest that LOX-1 plays a role in inflammation and immune response. Our observations indicated that hsCRP, TNF- α , IL6, and leptin were significantly reduced, as was sLOX-1, and changes in sLOX-1 were correlated with hsCRP and TNF- α . From these results, we speculate that inflammatory conditions were ameliorated by weight reduction. It follows that circulating sLOX-1 decreased. LOX-1 induction may therefore be associated with inflammation and immune response.

Recent reports [27-29] indicate that the basal expression of LOX-1 in endothelial cells is very low. However, it can be rapidly induced by pro-inflammatory, pro-oxidant, and mechanical stimuli such as oxLDL [27], TNF- α [25], hsCRP [28], and shear stress [29]. LOX-1 binding to oxLDL rapidly elevates reactive oxygen species (ROS) levels including superoxide anion and hydrogen

peroxide via a membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.

ROS can activate two signal transduction pathways involving either P38 mitogen-activated protein

kinase (MAPK) or phosphoinositide 3-kinase (PI3K), with both causing nuclear factor-kappa B

(NF- κ B) activation and enabling nuclear translocation and subsequent regulation of

5 pro-inflammatory gene expression [30-33]. According to our study, in which changes in serum

sLOX-1 levels were correlated with hsCRP and TNF- α , circulating sLOX-1 levels may be the result

of hsCRP and TNF- α activating LOX-1 via the NF- κ B system. Two subjects showed slight increases

in serum sLOX-1 levels, and their basal levels of sLOX-1 (7.1pg/ml, 11.0pg/ml), hsCRP (0.5mg/L,

0.7mg/L), and TNF- α (0.1pg/ml, 0.2pg/ml) were very low. Thus, these data may support this

10 phenomenon.

Two limitations of this study were the small sample size and the fact that subjects were comprised solely of middle- aged Japanese men. Future research should therefore make use of a

wider range of subjects in terms of age, gender, and race. Nevertheless, this is the first study to

examine whether serum sLOX-1 level can be related to weight reduction in over-weight individuals.

Further studies are needed to clarify the clinical evidence for sLOX-1 being useful as an early

biomarker for ACS.

5 In conclusion, serum sLOX-1 level was significantly reduced via weight reduction intervention

in middle-aged men. It was also significantly correlated with changes in BMI, fat mass, abdominal

fat area, serum lipids, leptin, hsCRP, and TNF- α . These results suggest that LOX-1 induction may be

related to adipocyte metabolism, inflammation, and immune response.

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Figure Legends

Comparison of changes in serum LOX-1 levels with changes in fat mass (FM) and high sensitive

CRP (hsCRP) by simple linear regression analyses (n = 32). A) shows Δ sLOX-1 vs. Δ FM B)

shows Δ sLOX-1 vs. Δ hsCRP. Serum sLOX-1 showed significant correlation with fat mass (r =

5 0.570, $P = 0.002$) and hsCRP (r = 0.564, $P = 0.002$). Only two subjects showed slight increases in

serum sLOX-1 levels.

Table 1

Subject characteristics at baseline and changes in measurements (n = 32)

	baseline	change	percent change	
Age (years)	50.3 ± 12.4			
Body weight (kg)	75.4 ± 6.4	-7.5 ± 4.8	-9.9 ± 5.8	***
Body mass index (kg/m ²)	26.8 ± 2.2	-2.6 ± 1.7	-9.7 ± 6.0	***
Fat mass (kg)	18.9 ± 4.0	-3.6 ± 2.9	-19.0 ± 15.6	***
% Fat mass (%)	25.1 ± 4.6	-2.7 ± 2.8	-11.0 ± 12.8	***
Visceral fat area (cm ²)	168.1 ± 57.7	-57.2 ± 44.7	-34.2 ± 23.8	***
Subcutaneous fat area (cm ²)	182.4 ± 56.9	-64.9 ± 37.6	-35.6 ± 18.1	***
Total fat area (cm ²)	350.4 ± 89.8	-122.1 ± 77.3	-34.2 ± 19.5	***
Systolic blood pressure (mmHg)	131.1 ± 26.8	-9.3 ± 11.3	-6.5 ± 8.3	***
Diastolic blood pressure (mmHg)	80.9 ± 17.5	-5.8 ± 8.6	-6.3 ± 10	***
TC (mg/dL)	216.2 ± 41.0	-13.5 ± 28.6	-6.2 ± 12.6	*
HDLc (mg/dL)	55.6 ± 10.4	6.2 ± 10.1	11.2 ± 23.5	**
LDLC (mg/dL)	134.0 ± 35.6	-10.8 ± 23.9	-8.1 ± 16.2	*
Triglycerides (mg/dL)	132.2 ± 55.6	-44.8 ± 56.9	-33.8 ± 48.9	***
Fasting glucose (mg/dL)	96.1 ± 21.5	-2.3 ± 12	-1.2 ± 13.1	
Insulin (μU/mL)	11.3 ± 2.7	-0.9 ± 6.1	-8.0 ± 14.6	
hemoglobin A1c (%)	5.3 ± 1.1	-0.3 ± 0.5	-4.3 ± 7.9	**
Maximal oxygen uptake (ml/kg/min)	29.3 ± 6.7	2.7 ± 4.6	9.6 ± 16.6	**
sLOX-1 (pg/ml)	33.8 ± 24.7	-24.3 ± 23.2	-72.1 ± 35.9	***
high sensitive CRP (mg/L)	1.4 ± 1.8	-0.5 ± 1.7	-34.5 ± 110.0	*
TNF-α (pg/ml)	0.7 ± 0.2	-0.1 ± 0.2	-10.1 ± 29.0	*
Interleukin-6 (pg/ml)	2.2 ± 1.5	-0.7 ± 1.5	-7.6 ± 76.3	**
Leptin (ng/ml)	6.5 ± 4.4	-3.7 ± 4.0	-57.4 ± 4.8	**
adiponectin (μg/ml)	4.1 ± 1.5	1.8 ± 1.7	43.9 ± 40.8	***

Data are mean ± SD. Percent change = change / baseline × 100. TC, total cholesterol; HDLC, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1; CRP, C-reactive protein; TNF, tumor necrosis factor.

P* < 0.05, *P* < 0.01, ****P* < 0.001.

Table 2

Correlation coefficients at baseline and changes in sLOX-1 (n = 32)

	baseline	change
Body weight (kg)	r = 0.473 *	r = 0.542 **
Body mass index (kg/m ²)	r = 0.453 *	r = 0.574 ***
Fat mass (kg)	r = 0.343	r = 0.570 **
Visceral fat area (cm ²)	r = -0.135	r = 0.252
Subcutaneous fat area (cm ²)	r = 0.319	r = 0.498 **
TC (mg/dL)	r = 0.275	r = 0.405 *
Triglycerides (mg/dL)	r = 0.361	r = 0.219
HDLC (mg/dL)	r = -0.213	r = -0.254
LDLC (mg/dL)	r = 0.276	r = 0.489 **
Fasting glucose (mg/dL)	r = -0.328	r = 0.097
hemoglobin A1c (%)	r = -0.281	r = 0.022
Maximal oxygen uptake (ml/kg/min)	r = 0.053	r = -0.129
high sensitive CRP (mg/L)	r = 0.575 ***	r = 0.564 **
TNF- α (pg/ml)	r = 0.263	r = 0.321
Interleukin-6 (pg/ml)	r = 0.146	r = 0.196
Leptin (ng/ml)	r = 0.421 *	r = 0.468 *
adiponectin (μ g/ml)	r = -0.177	r = 0.176

TC, total cholesterol; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol;

5 sLOX-1, soluble lectin-like oxidized low-density lipoprotein receptor-1;

CRP, C-reactive protein; TNF, tumor necrosis factor.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3

Stepwise multiple regression analysis for change in sLOX-1 (n= 32)

Independent variable	Beta	<i>P</i>	Change in R ² (%)
Δ BMI	0.574	0.001	30.4
ΔhsCRP	0.128	0.006	15.5

Figure 1

