

An Experimental Study That Investigates Effects of Atreleuton in Metabolic Syndrome

Tevfik Noyan*, Sertaç Burhanettin Ayhan

Department of Biochemistry, Ordu University Medical Faculty, Ordu, Turkey

ABSTRACT

The inflammation, LDL oxidation, and insulin resistance play an important role in the development of metabolic syndrome (MetS). This study aims to investigate the effects of atreleuton on serum lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), leukotriene B4 (LTB-4), insulin, and adipose tissue glucose transporter 4 (GLUT-4) levels in an experimental MetS model in rats. The rats were randomly divided into 3 groups: Group 1 (control, n = 7); Group 2 (MetS group, n = 8), Group 3 (MetS+atreleuton administered group, atreleuton (25 mg/kg/day) was administered orally by gavage during the last 4 weeks of the experiment). MetS was created by feeding rats with high fructose and fat diet for 10 weeks in groups 2 and 3. Measurements of test parameters were made by ELISA method. The atreleuton caused a significant decrease in levels of serum LTB-4, LOX-1, insulin, and homeostatic model assessment for insulin resistance (HOMA-IR), while significantly increase on quantitative insulin sensitivity check index (QUICKI). However, atreleuton did not cause a significant change in tissue GLUT-4 level. The results of this study suggest that atreleuton may be a protective agent against MetS complications by decreasing the levels of LTB-4 and LOX-1 that play an important role in MetS pathogenesis. Besides, the effect of atreleuton on the tissue GLUT-4 pathway needs to be supported by further studies.

Keywords: Atreleuton, GLUT-4, insulin resistance, LTB-4, LOX-1, metabolic syndrome.

Introduction

Metabolic syndrome (MetS) is a complex condition characterized by hyperglycemia (insulin resistance), central obesity, high blood pressure, dyslipidemia, and microalbuminuria. Insulin resistance is an absolute requirement for the diagnosis of MetS, provided the two criteria mentioned above are met (1). MetS cause a significantly increased risk of coronary artery disease (CAD), type 2 diabetes mellitus (T2DM), stroke, and hepatic steatosis. Genetic variations, environmental factors, high-calorie dietary intake, and lack of exercise play an crucial role in MetS pathogenesis. As is known, fructose converts to glycerol, which is the precursor of triglyceride synthesis, so it is thought that excessive fructose consumption may be a critical factor in the development of MetS by causing excessive accumulation of ectopic fat in the liver (2).

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a type II transmembrane glycoprotein. LOX-1 is secreted from endothelial cells, vascular smooth muscle cells, macrophages, and adipocytes. It leads to endothelial dysfunction, apoptosis, and atherosclerotic process through intracellular signal transduction (3). LOX-1 causes the oxidation of LDL, leading to the development of various metabolic, cardiovascular, and other diseases. It has been

reported that the LOX-1 level also increases in MetS (4).

Insulin stimulates glucose uptake into skeletal muscle and adipose tissue via glucose transporter 4 (GLUT-4). Decreasing GLUT-4 activity on skeletal and adipose tissue cell surfaces contributes to insulin resistance. Conversely, overexpression of GLUT-4 in adipocytes reduces fasting glycemia and improves glucose tolerance (5). The development of insulin resistance and inflammation is the most widely accepted hypothesis for MetS formation (6).

Leukotrienes (LTs) are mediators of inflammation, termed eicosanoids, which are synthesized from arachidonic acid and released from cell membrane by the enzyme phospholipases. There are several types of LTs and one of them is leukotriene B4 (LTB-4). LTB-4 is the strongest chemotactic agent for neutrophils and plays a crucial role in many inflammatory diseases. LTB-4 acts through two membrane receptors; BLT1 and BLT2 (7). LTB-4 causes secretion of adipokine from obese adipose tissue and contributes to obesity-related inflammation and lipid dysfunction (8).

Atreleuton (1-[(2R)-4-[5-[(4-fluorophenyl) methyl] thiophen-2-yl] but-3-yn-2-yl]-1-hydroxyurea. C₁₆H₁₅FN₂O₂S, VIA-2291), a selective redox inhibitor, inhibits the 5-lipoxygenase enzyme (5-LO).

*Corresponding Author: Tevfik Noyan, Ordu University, Medical Faculty, Department of Biochemistry, Ordu, 52200, Türkiye

E-mail: tnoyan@odu.edu.tr, tevfiknoyan@hotmail.com, Phone: +90 (452) 226 52 07, Fax : +90 (452) 226 52 08

ORCID ID: Tevfik Noyan: 0000-0002-7733-0177, Sertaç Burhanettin Ayhan: 0000-0002-1915-1306

Received: 06.10.2020, Accepted: 01.02.2021

The beneficial effects of atreleuton have been proven in some studies, especially in clinical conditions accompanied by inflammation such as atherosclerosis and cardiovascular diseases (9). It has been reported that atreleuton might reduce leukotriene B4 and leukotriene E4 levels in humans (10).

The present study, it was aimed to investigate the effects of atreleuton on LOX-1, LTB-4 GLUT-4, insulin resistance and lipid parameters on an experimental MetS model in rats. To our knowledge, this is the first study investigating the effects of atreleuton on MetS.

Materials and Method

All experimental protocols followed the principles of the Declaration of Helsinki and were carried out by the European Communities Council directive (86/609/EEC). The study procedure was approved with 11/02/2015 dated and 82678388/16 numbered decision by Experimental Animals Local Ethics Committee. In the present study, a total of 23 Sprague Dawley female rats with body weights between 125-150 grams were used. Before the study, the rats were housed in standard cages and released from feed and water intake. The rats were housed at 22 ± 2 °C for 12 hours in darkness and 12 hours in light. Rats in the control group continued normal nutrition with no procedure performed. MS was induced by a modified method (11). The rats were fed with a standard rodent chow in addition to 25% fat, 3% NaCl, and fructose 35% solution in drinking water. Diet and fructose solutions were freshly prepared every day. The rats were randomly divided into three groups:

Group 1 (control, n=7); a normal laboratory diet, tap water ad libitum and saline were given to rats daily for 10 weeks.

Group 2 (MetS group, n=8); a high-fructose high-fat diet (HFHFD), and saline were given to rats daily for 10 weeks.

Group 3 (MetS+atreleuton group, n=8); HFHFD and saline were given to rats daily for 10 weeks. Atreleuton (25 mg/kg/day) was administrated orally by gavage during the last 4 weeks of the experiment.

Changes in the body weight of rats were noted individually. At the end of the experiment, the animals were fasted for 12 hours weighed and blood samples were withdrawn from the retro-orbital plexus under light anesthesia. After being kept at room temperature for 30 min, blood samples were centrifuged at 1800*g for 15 min. Serum samples were divided into small aliquots and stored in a deep freezer at -80 °C for 3 months for the estimation of the levels of LOX-1, LTB-4, insulin, aspartate

aminotransaminase (AST), alanine aminotransaminase (ALT), triglyceride (TG), total cholesterol (TC), and total protein.

Animals were killed by cervical dislocation, visceral adipose tissue was rapidly excised, washed with saline, weighed, and homogenized in ice-cold for GLUT-4 and total protein measurements. The homogenates were prepared on ice in the ratio 1:4 (weights: volume), 0.1 M phosphate, 0.1 mM EDTA, pH 7.0 in homogenization buffer.

LOX-1, LTB-4, insulin, and GLUT-4 levels were measured by a commercial ELISA kit (Bioassay Technology Laboratory) according to the manufacturer's instruction. The concentrations were calculated by comparing the optical density of the samples to the standard curves. Serum AST, ALT, triglyceride, total cholesterol, and protein measurements were performed colorimetrically on autoanalyzer (Hitachi PP Modular) by using commercial kits (ARCHITECT).

QUICKI (quantitative insulin sensitivity check index) and HOMA-IR (homeostasis model assessment - insulin resistance) values were calculated based on fasting glucose (G0) and insulin (I0) levels using the following formulas: $HOMA-IR = (G0 \times I0)/22.5$; $QUICKI = 1/[\log(G0) + \log(I0)]$ (12).

Statistical Analysis: The power analysis was performed to determine the minimum sample size before starting the study. Normality assumption of the variables was tested with Kolmogorov-Smirnov test and homogeneity control of groups was performed by the Levene test. The data for the weight variable levels of one of the two-factor factors were evaluated by repeated measures variance analysis (Two-way ANOVA with repeated measures on one factor) and the remaining variables were evaluated by one-way variance (One-way ANOVA) analysis. Tukey multi-comparison test was performed at 5% significant level to determine different groups. The results were presented as mean \pm standard deviation (SD).

Results

The weight values of the groups are shown in Table 1. There were no significant differences among all groups at the beginning of the study. At the end of the study, significantly increased average body weight was found in all groups compared to their beginning values, the weight averages of the rats in groups 2 and 3 were also significantly higher than group 1. However, the weight averages of the rats in group 3 were also significantly lower than group 2.

The descriptive statistical values of biochemical

Table 1. The comparison of initial and final weights of rats in the groups

Grup	n	Initial		Final	
		Mean	SD	Mean	SD
Group 1	7	130.14 Ab	8.91	272.85 Ca	9.12
Group 2	8	139.12 Ab	10.75	341.62 Aa	11.34
Group 3	8	135.25 Ab	11.70	328.87 Ba	7.68
p		Group × Time : <0.001			

Two-way ANOVA with repeated-measures on one factor and Tukey's HSD test. The difference between group means without common capital letters in the same column is significant ($p < 0.05$). The difference between time averages without common small letters in the same row is statistically significant ($p < 0.05$)

Table 2. Descriptive statistical analysis and comparison of results for LTB-4, LOX-1 and tissue GLUT-4 levels in the groups (LTB-4; leukotriene B4. LOX-1; lectin-like oxidized low-density lipoprotein receptor-1, GLUT-4; glucose transporter 4).

	Group 1	Group 2	Group 3	P
	Mean ± SD	Mean ± SD	Mean ± SD	
LTB-4 (ng/L)	0.73±0.07b	0.98±0.06a	0.67±0.04b	<0.001
LOX-1 (ng/mL)	0.87±0.24c	2.44±0.27a	1.85±0.36b	<0.001
GLUT-4 (ng/g protein)	9.93±2.61b	24.92±8.36a	19.37±6.93a	0.001**

One-way ANOVA and Tukey's HSD test, **, $p < 0.01$. The difference between means without a common letter is statistically significant ($p < 0.05$).

Table 3. Descriptive statistical analysis and comparison of results for other biochemical parameters in the groups (HOMA-IR; insulin resistance index. QUICKI; quantitative insulin sensitivity check index, AST; aspartate aminotransaminase, ALT; alanine aminotransaminase).

	Group 1	Group 2	Group 3	p
	Mean ± SD	Mean ± SD	Mean ± SD	
Insulin (μIU/L)	5.57±0.65b	12.14±3.02a	7.44±2.06 b	<0.001
Glucose (mg/dL)	122.71±6.40c	152.25±8.60a	142.62±6.16b	<0.001
HOMA-IR	1.69±0.24b	4.54±1.07a	2.61±0.72b	<0.001
QUICKI	0.35±0.00a	0.31±0.01c	0.33±0.01b	0.001**
AST (U/L)	185.57±36.61	258.62±153.38	201.38±49.20	0.323
ALT (U/L)	49.14±18.17	72.62±31.77	64.88±27.90	0.257
Cholesterol (mg/dL)	40.29±10.54	51.88±15.76	41.5±5.73	0.117
Triglycerid (mg/dL)	55.43±12.28c	136.38±28.00a	85.25±7.48b	<0.001
Total protein (g/L)	5.70±0.78	5.73±1.09	4.71±1.08	0.097

One-way ANOVA and Tukey's HSD test, **, $p < 0.01$.

The difference between means without a common letter is statistically significant ($p < 0.05$).

variables and comparison of obtained results are presented in Tables 2 and 3. As shown in these Tables, the serum level of LTB-4 was significantly increased in Group 2 compared to Groups 1 and 3, while there was no statistically significant difference between Groups 1 and 3.

Serum LOX-1 level increased significantly in both Groups 2 and 3 compared to group 1 and decreased significantly in the group 3 compared to group 2.

Tissue GLUT-4 level showed a statistically significant increase in the Group 2 and 3 compared to Group 1,

but there was no significant difference between the Group 2 and 3.

Compared to Groups 1 and 3, a significant increase in serum insulin, glucose, and HOMA-IR levels, and decrease in QUICKI value were obtained in the Group 2. Also, significantly increased glucose level and decreased QUICKI value were found in the Group 3 compared to Group 1.

The serum triglyceride level was significantly increased in Group 2 and 3 as compared to Group 1, and decreased in the Group 3 compared to Group 2. There was no significant difference among the groups

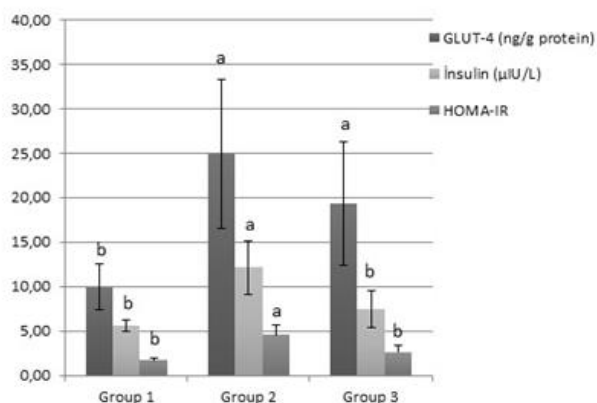


Fig. 1. Comparison of GLUT-4, insulin and HOMA-IR levels in the groups (GLUT-4; glucose transporter 4, HOMA-IR; insulin resistance index). The difference between means without a common letter is statistically significant ($p < 0.05$).

in terms of serum protein, cholesterol levels, and AST and ALT activities.

The comparison levels of LTB-4, LOX-1, GLUT-4, insulin, HOMA-IR, and QUICKI among the groups are presented in Figures 1 and 2.

Discussion

Numerous animal models are developed for the investigation of MetS, however, rats and mice are the most commonly used for this aim. The use of a single type or a combination of diets, such as high-fructose, high-sucrose, high-fat, high-fructose/high-fat, or high-sucrose/high-fat diets were capable to induce MetS in animals (13). In this experimental study, a high-fat and high-fructose diet was chosen to induce MetS. As known, fructose over-consuming leads to the accumulation of triglycerides and cholesterol into the liver, subsequently causing insulin resistance and glucose intolerance.

The obesity and metabolic disorders are characterized by low-grade inflammation. LTB-4 is the most potent inflammatory mediator and leads to the production of adipokines such as monocyte chemoattractant protein (MCP-1) and interleukin (IL)-6 from obese adipose tissue. Moreover, the increase in the activity of the LTB-4-BLT1 pathway has been reported to cause insulin resistance in obese mice (14, 15). To inhibit LTB-4 biosynthesis, suppression of phospholipases enzyme and inhibition of 5-LOX are among the preferred pharmacological strategies (7).

Atreleuton is an iron-chelating agent, a selective and reversible inhibitor of 5-LO, a key enzyme in LTs biosynthesis. In this study, administration of Atreleuton caused a significant decrease in LTB-4 levels, which increased due to MetS. To date, no

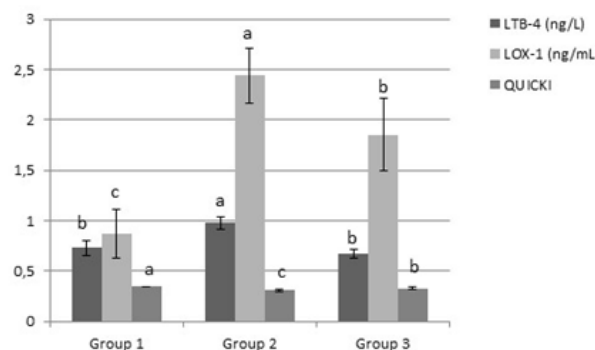


Fig. 2. Comparison of LTB-4, LOX-1, and QUICKI levels in the groups (LTB-4; leukotriene B4, LOX-1; lectin-like oxidized low-density lipoprotein receptor-1, QUICKI; quantitative insulin sensitivity check index). The difference between means without a common letter is statistically significant ($p < 0.05$).

study investigates the relation of Atreleuton and LTB-4 in MetS. The stimulation of 5-LO pathway caused the secretion of inflammatory leukotrienes and they have a promoting role in the pathogenesis of atherosclerosis and aortic aneurysms (16). The blockage of 5-LO pathway is a crucial target for drug development for some diseases (17). The hypercholesterolemia caused to increase in LTB-4 levels in neutrophils (18). It was showed that Atreleuton caused a reduction in LTB-4 production in patients with acute coronary syndrome (10). In contrast to these findings, it was reported that Atreleuton did not have any effect on the reduction of arterial inflammation (19). This study is the first to show the inhibitory effects of Atreleuton on LTB-4 in MetS. We think that further studies are required to investigate the relationship between Atreleuton and MetS complications.

Increased LOX-1 levels have a crucial role in the progression of vascular inflammation, atherosclerotic plaque formation, destabilization, erosion, and rupture (20). Previous studies reported that increased LOX-1 levels are a critical role in metabolic disorders such as obesity, T2DM, and other medical conditions closely related to MetS (21-23). Our results suggest that atreleuton might have the potential to reverse the damage in MetS via a decrease in LOX-1 levels. The proteolytic destruction of LOX-1 from the cell-membrane-proximal extracellular domain leads to the release of soluble receptors such as sLOX-1 (24). The level of circulating soluble receptors may reflect the expression of membrane proteins; hence, sLOX-1 might be a potential biomarker of vascular diseases. LOX-1 expression is low under normal physiological conditions. However, the presence of inflammatory conditions caused an upregulation of LOX-1 gen. As similar to our results, some clinical studies reported LOX-1 has a potential role in the development of

MetS (22, 23). It was suggested that circulating plasma sLOX-1 may be able to bind ox-LDL, and act as a decoy for ox-LDL (25). It has been reported that many drugs that inhibit LOX-1 cause a decrease in damage due to oxidative stress (26). It has shown that antibody administration that inhibits LOX-1 in vitro reverses the lesions in the nephropathy of obese rats with diabetes and dyslipidemia (27). To our knowledge, our study is the first to reveal the relationship between atreleuton and LOX1 in MetS. The results of this study show that atreleuton significantly lowers serum sLOX-1 levels. To our knowledge, there are no known drugs that directly inhibit LOX-1. Therefore, we think that this study needs to be supported by more studies.

The uptake of glucose from the blood inside the skeletal muscle and adipose tissue cells is performed by the insulin-dependent mechanism. GLUT-4 is a glucose transporter protein in these tissues. Insulin stimulates the translocation of GLUT-4 from the intracellular compartment to the cell membrane (28). In the present study, the administration of Atreleuton to MS-induced rats provoked a significant reduction of blood glucose associated with the reduction of insulin level, and HOMA index, and as well as increased QUICKI value. However, neither the presence of MetS nor the application of atreleuton caused a significant change in tissue GLUT 4 level. MetS caused an increase in serum triglyceride levels in rats, while administration of Atreleuton resulted in a significant decrease in blood triglyceride levels. The results of the present study indicate that atreleuton might increase glucose uptake and insulin sensitivity. The stimulation of GLUT-4 expression and translocation is essential for the maintenance of glucose homeostasis in the management of insulin resistance and T2DM. It was known that the absence of GLUT-4 resulted in reduced insulin-stimulated glucose transport in adipocytes (29). There are currently no treatments that directly increase glucose transport in muscle and adipose tissue, which might confer more metabolic benefits beyond glucose metabolism (30). Therefore, we think that it is necessary for more studies that investigate the association of atreleuton on tissue GLUT-4 levels.

In conclusion, the results of our study suggest that atreleuton might be an anti-inflammatory and anti-oxidant agent in MetS via the down-regulate of LTB-4 and sLOX. Also, atreleuton caused to decrease in insulin resistance, serum glucose, and triglyceride levels. The possible effects of atreleuton on tissue GLUT-4 pathway are limited. We believe that large-scale experimental and clinical studies are required to fully address whether Atreleuton might have a therapeutic benefit in the treatment of MetS.

Declaration of conflicting interests: The author(s) declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Acknowledgments: The authors would like to thank Assistant Professor Yeliz Kasko Arıcı, Ordu University, Ordu/ Turkey, for helping statistical analysis.

Financial Disclosure: This study was supported by Ordu University Scientific Research Projects Coordination Department (Project Number: AR-1667).

References

1. Xu H, Li X, Adams H, Kubena K, Guo S. Etiology of metabolic syndrome and dietary intervention. *Int J Mol Sci* 2019; 20: 128.
2. Mellen PB, Cefalu WT, Herrington DM. Diabetes, the metabolic syndrome, and angiographic progression of coronary arterial disease in postmenopausal women. *Arterioscler Thromb Vasc Biol* 2006; 26: 189-193.
3. Ozturk O, Colak Y, Senates E, et al. Increased serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels in patients with biopsy-proven nonalcoholic fatty liver disease. *World J Gastroenterol* 2015; 21: 8096-8102.
4. Md Sayed AS, Zhao Z, Guo L, et al. Serum lectin-like oxidized-low density lipoprotein receptor-1 and adiponectin levels are associated with coronary artery diseases accompanied with metabolic syndrome. *Iran Red Crescent Med J* 2014; 16: 12106.
5. Klip A, McGraw TE, James DE. Thirty sweet years of GLUT-4. *J Biol Chem* 2019; 294: 11369-11381.
6. Leguisamo NM, Lehnen AM, Machado UF, et al. GLUT-4 content decreases along with insulin resistance and high levels of inflammatory markers in rats with metabolic syndrome. *Cardiovasc Diabetol* 2012; 11: 100.
7. Wan M, Tang X, Stsiapanava A, Haeggström JZ. Biosynthesis of leukotriene B4. *Semin Immunol* 2017; 33: 3-15.
8. Filgueiras LR, Serezani CH, Jancar S. Leukotriene B4 as a potential therapeutic target for the treatment of metabolic disorders. *Front Immunol* 2015; 515.
9. Back M. Inhibitors of the 5-lipoxygenase pathway in atherosclerosis. *Curr Pharm Des.* 2009, 15: 3116-32.
10. Tardif JC, L'allier PL, Ibrahim R, et al. Treatment with 5-lipoxygenase inhibitor VIA-2291 (Atreleuton) in patients with recent

- acute coronary syndrome. *Circ Cardiovasc Imaging* 2010; 3: 298-307.
11. Calvo-Ochoa E, Hernández-Ortega K, Ferrera P, Morimoto S, Arias C. Short-term high-fat-and-fructose feeding produces insulin signaling alterations accompanied by neurite and synaptic reduction and astroglial activation in the rat hippocampus. *J Cereb Blood Flow Metab* 2014; 1001-1008.
 12. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; 85: 2402-2410.
 13. Wong SK, Chin KY, Suhaimi FH, Fairus A, Ima-Nirwana S. Animal models of metabolic syndrome: a review. *Nutr Metab* 2016; 13: 65.
 14. Chakrabarti SK, Wen Y, Dobrian AD, et al. Evidence for activation of inflammatory lipoxygenase pathways in visceral adipose tissue of obese Zucker rats. *Am J Physiol Endocrinol Metab* 2011; 300: 175-187.
 15. Li P, Oh DY, Bandyopadhyay G, et al. LTB-4 promotes insulin resistance in obese mice by acting on macrophages, hepatocytes and myocytes. *Nat Med* 2015; 21: 239-247.
 16. De Caterina R and Zampolli A. From asthma to atherosclerosis-5-lipoxygenase, leukotrienes, and inflammation. *N Engl J Med* 2004; 350: 47.
 17. Pettersen D, Broddefalk J, Emtenaes H, et al. Discovery and early clinical development of an inhibitor of 5-Lipoxygenase activating protein (AZD5718) for treatment of coronary artery disease. *J Med Chem* 2019; 62: 4312-4324.
 18. Lai XF, Qin HD, Guo LL, Luo ZG, Chang J, Qin CC. Hypercholesterolemia increases the production of Leukotriene B4 in neutrophils by enhancing the nuclear localization of 5-Lipoxygenase. *Cell Physiol Biochem* 2014; 34: 1723-1732.
 19. Gaztanaga J, Farkouh M, Rudd JH, et al. A phase 2 randomized, double-blind, placebo controlled study of the effect of VIA-2291, a 5-lipoxygenase inhibitor, on vascular inflammation in patients after an acute coronary syndrome. *Atherosclerosis* 2015; 240: 53-60.
 20. 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.
 21. Brinkley TE, Kume N, Mitsuoka H, Phares DA, Hagberg JM. Elevated soluble lectin-like oxidized LDL receptor-1 (sLOX-1) levels in obese postmenopausal women. *Obesity (Silver Spring)* 2008; 16: 1454-1456.
 22. Puttaruk P, Pipatsatitpong D, Siripurkpong P. Soluble lectin-like oxidized low density lipoprotein receptor-1 in metabolic syndrome. *Asian Biomed (Res. Rev. News)* 2015; 9: 675-682.
 23. Civelek S, Kutnu M, Uzun H, et al. Soluble lectin-like oxidized LDL receptor 1 as a possible mediator of endothelial dysfunction in patients with metabolic syndrome. *J Clin Lab Anal* 2015; 29: 184-190.
 24. Murase T, Kume N, Kataoka H, et al. Identification of soluble forms of lectin-like oxidized LDL receptor-1. *Arterioscler Thromb Vasc Biol* 2000; 20: 715-720.
 25. Navarra T, Del Turco SD, Berti S, Basta G. The lectin-like oxidized low density lipoprotein receptor-1 and its soluble form: cardiovascular in applications. *J Atheroscler Thromb* 2010; 17: 317-331.
 26. Li Q, Zhao W, Zeng X, Hao Z. Ursolic acid attenuates atherosclerosis in ApoE (-/-) mice: role of LOX-1 mediated by ROS/NF-kappa B pathway. *Molecules* 2018; 23: 1101.
 27. Dominguez JH, Mehta JL, Li D. et al. Anti-LOX-1 therapy in rats with diabetes and dyslipidemia: ablation of renal vascular and epithelial manifestations. *Am J Physiol Renal Physiol* 2008; 294: 110-119.
 28. Zayed EA, AinShoka. AA, El Shazly KA, Abd El Latif HA. Improvement of insulin resistance via increase of GLUT-4 and PPARγ in metabolic syndrome-induced rats treated with omega-3 fatty acid or l-carnitine. *J Biochem Mol Toxicol* 2018; 32: 22218.
 29. Abel ED, Peroni O, Kim JK, et al. Adipose-selective targeting of the GLUT-4 gene impairs insulin action in muscle and liver. *Nature* 2001; 409: 729-733.
 30. Fazakerley DJ, Krycer JR, Kearney AL, Hocking SL, James DE. Muscle and adipose tissue insulin resistance: a malady without mechanism? *J Lipid Res* 2019; 60: 1720-1732.