repiSalud
Repositorio Institucional en Salud

This is the peer reviewed version of the following article:

Hurtado-Roca Y, Bueno H, Fernandez-Ortiz A, Ordovas JM, Ibanez B, Fuster V, et al. *Oxidized LDL is Associated with Metabolic Syndrome Traits Independently of Central Obesity and Insulin Resistance*. Diabetes. 2017;66(2):474-82

which has been published in final form at: https://doi.org/10.1016/j.jacc.2017.05.033

Title: Oxidized LDL is associated with metabolic syndrome traits independently of central obesity and insulin resistance

Yamilee Hurtado-Roca MD[1,2,3], Hector Bueno MD PhD[1,4], Antonio Fernandez-Ortiz MD PhD[1,5], Jose Maria Ordovas PhD[1,6], Borja Ibañez MD PhD[1,7], Valentin Fuster MD PhD[1,8], Fernando Rodriguez-Artalejo MD PhD[2], Martin Laclaustra MD PhD[1,2,9]

[1] Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain

[2] CIBERESP and Department of Preventive Medicine and Public Health, School of Medicine, Universidad Autonoma de Madrid/Idipaz, Madrid, Spain.

[3] Boca Raton Clinical Research Global Peru, Lima, Peru.

[4] Hospital 12 de Octubre, Madrid, Spain.

[5] Hospital Clínico San Carlos, Universidad Complutense, Madrid, Spain.

[6] US Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, MA, United States.

[7] IIS-Fundación Jiménez Díaz Hospital, Universidad Autónoma, Madrid, Spain.

[8] Icahn School of Medicine at Mount Sinai, New York, NY, United States.

[9] Department of Epidemiology, St. Louis University, St Louis, MO, United States.

Corresponding author:

Martin Laclaustra, MD, PhD, MPH

CIBERESP and Department of Preventive Medicine and Public Health, School of Medicine

Universidad Autonoma de Madrid

Arzobispo Morcillo, 4, 28029 Madrid, Spain

Phone: +34 34 91 497 5441

E-Mail: martin.laclaustra@uam.es

ABSTRACT

This study assesses whether oxidative stress, using oxidized LDL (ox-LDL) as proxy, is associated with metabolic syndrome (MS), whether ox-LDL mediates the association between central obesity and MS, and whether insulin resistance mediates the association between ox-LDL and MS. We examined baseline data from 3987 non-diabetic subjects in the Progression of Early Subclinical Atherosclerosis (PESA) Study. For the 2nd, 3rd, and 4th ox-LDL quartiles versus the 1st, the odds ratios (95% confidence interval) for MS were 0.84 (0.52, 1.36), 1.47 (0.95, 2.32), and 2.57 (1.66, 4.04) ($p<0.001$ for trend) once adjusted for age, sex, smoking, LDL-cholesterol, body mass index, waist circumference, and HOMA-IR. Results showing the same trend were found for all MS components except glucose concentration. Ox-LDL mediated 13.9 % of the association of waist circumference with triglycerides and only 1-3% of the association with HDL-cholesterol, blood pressure, and insulin concentration. HOMA-IR did not mediate the association between ox-LDL and MS components. In this study, higher ox-LDL concentrations were associated with MS and its components independently of central obesity and insulin resistance. Ox-LDL may reflect core mechanisms through which MS components develop and progress in parallel with insulin resistance and could be a clinically relevant predictor of MS development.

Clinical Trial Registration—URL: http://www.clinicalTrials.gov. Unique identifier: NCT01410318

Key words: oxidized-LDL, metabolic syndrome, insulin resistance, and obesity

Increased oxidative stress is the consequence of an imbalance between oxidant and antioxidant biological agents and can result in damage to biomolecules, including proteins, nucleic acids, and lipids. Some of these damaged biomolecules have been used as oxidative stress biomarkers, such as oxidized-low density lipoproteins (ox-LDL)(1), that can be measured from a regular blood draw.

Oxidative stress is suspected to be involved in the pathophysiology of several chronic diseases(2,3) and has been linked to metabolic syndrome (MS), a cluster of risk factors for cardiovascular disease that includes central obesity, high blood pressure, high fasting glucose, and dyslipidemia(4–7). A common hallmark of MS-associated dyslipidemia is elevation of small and dense low-density lipoprotein (LDL) particles, which are easily oxidized(8). Also, high ox-LDL levels are associated with insulin resistance(9), which is tightly linked to the pathogenesis of MS(10). Insulin resistance may arise from oxidative-stress-mediated activation of kinase signaling cascades that phosphorylate insulin receptors, leading to impaired insulin action(11); but it is also related to the correlation between plasma glucose and LDL susceptibility to oxidation(12). Indeed, high glucose concentrations might even induce LDL oxidation(13,14). In addition, obesity is the main origin of MS and it has been involved with induction of oxidative stress(15), which in turn may contribute to the development of MS(16).

However, no previous study has analyzed with detail which elements mediate the associations between obesity, oxidative stress, insulin resistance, and MS in humans. Using data from the Progression of Early Subclinical Atherosclerosis (PESA) study(17), which is a carefully phenotyped sample with size big enough to address subtle associations, this study aims to assess 1) whether oxidative stress, using ox-LDL as a proxy, is associated with MS, 2) whether ox-LDL mediates the association between central obesity and MS, and 3) whether

insulin resistance mediates the association between ox-LDL and MS (Supplementary Figure 1).

RESEARCH DESIGN AND METHODS

Study design and population

We used baseline data from PESA(17), a prospective cohort study aimed to evaluate traditional and novel risk factors and atherosclerosis in the carotid, aortic, coronary and iliofemoral territories using accessible noninvasive imaging techniques(18) in asymptomatic male and female employees (40-54 years old) of the Banco Santander in Madrid (Spain) who were free of clinical atherosclerosis. All participants were recruited between 2010 and 2013. The PESA study was approved by the Ethics Committee of the Instituto de Salud Carlos III in Madrid, the study protocol was conducted according to the guidelines of The Helsinki Declaration, and all participants gave written informed consent.

From an initial sample of 4117 participants, we excluded 82 with diabetes, 2 with missing ox-LDL data, and 46 with no recorded smoking status. Data was complete for all other relevant variables. The final analytical sample thus included 3987 individuals.

Data collection

Data were obtained from structured clinical interviews and questionnaires, a physical exam, and a fasting blood sample. With the patient standing, waist circumference was measured at a midpoint plane between the iliac crest and the costal border. Weight was measured without shoes and outdoor clothes to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm, again without shoes and with participants standing upright with their back to the stadiometer. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2); overweight was defined as BMI between 25 and 30, and obesity as

BMI≥30(19). Blood pressure was calculated as the mean of three consecutive measurements made with an automatic oscillometric OMRON HEM-907 sphygmomanometer (OMRON Healthcare Co. Ltd., Kyoto, Japan), which has been validated according to international protocols(20); participants were seated for 5 minutes before the measurements were made, and blood pressure readings were made at 1-minute intervals. All procedures were ISO-9001 certified.

Laboratory measurements

Peripheral venous blood was collected after an 8-hour fast. A monoclonal antibody 4E6–based competition ELISA (Mercodia AB, Sweden) was used for measuring plasma levels of ox-LDL. Monoclonal antibody 4E6 is directed against a conformational epitope in the apoB-100 moiety of LDL that is generated as a consequence of substitution of lysine residues of apoB-100 with aldehydes. Whole blood HbA1c was measured by reverse-phase cationic exchange chromatography and double wavelength colorimetric quantification (BIORAD D-10, D-10™ Hemoglobin Testing System). Triglycerides, total cholesterol, HDL-cholesterol, and glucose were measured in serum with spectrophotometric assays in the Architect-Ci8200 analyzer, using the manufacturer's kits (Instrumentation Laboratory). Insulin was determined in the same analyzer by chemiluminescence immunoassay. Low-density lipoprotein cholesterol (LDL-cholesterol) was calculated from the Friedewald equation.

Metabolic syndrome and insulin resistance

According to the 2009 harmonizing definition(21), MS was diagnosed when participants met at least 3 of the following 5 criteria: high waist circumference (≥102 cm in men and ≥88 cm in women), high triglycerides (≥1.7 mmol/L, i.e. ≥150 mg/dL), low high-density lipoprotein cholesterol (HDL-cholesterol) (<1.0 mmol/L, i.e. <40 mg/dL, in men and

<1.3 mmol/L, i.e. <50 mg/dL in women), high blood pressure (≥ 130/85 mmHg or treatment with antihypertensive medication), and high fasting glucose (≥5.6 mmol/L, i.e. ≥100 mg/dL, or drug treatment for elevated glucose).

HOMA-IR (Homeostatic model assessment - Insulin Resistance) was calculated as glucose (mg/dL) multiplied by insulin (μU/mL) and divided by 405(22). Insulin resistance was defined as HOMA-IR ≥2.6(23).

Statistical analysis

Adjusted mean differences in metabolic variables across ox-LDL quartiles were calculated using linear regression analysis. Odds ratios (OR) and their 95% confidence interval (CI) were estimated with generalized linear models to quantify the association of quartiles of ox-LDL with the presence of MS, its components, insulin resistance, and metabolic clusters. Analyses used the first ox-LDL quartile as the reference group.

A basic model was adjusted for age (continuous), sex, smoking status, and LDL-cholesterol (continuous); we decided to include LDL-cholesterol in the basic model of adjustment because it is strongly associated with ox-LDL. The full model was adjusted for the variables in the basic model plus HOMA-IR (log-transformed), BMI (continuous), and waist circumference (continuous). Models including ox-LDL as a continuous variable were used to assess linear trend and to perform a bootstrapped mediation analysis.

As the full model included waist circumference (one of the variables used in the MS definition), we also evaluated the association of ox-LDL with clusters of non-anthropometric MS components: ≥2 or ≥3 criteria for MS other than high waist circumference.

Mediation analysis was used to analyze the extent to which ox-LDL explains the association of central obesity (measured by waist circumference) with MS components and

the extent to which HOMA-IR mediates the effect of ox-LDL on MS component values. The average direct effect, the average causal mediation effect, and the proportion of effect mediated with respect to the total effect were estimated by means of non-parametric bootstrapping with 1000 resamples and percentile-based confidence intervals(24).

Differences were considered statistically significant at $p < 0.05$. Statistical analyses were performed using R statistical software (version 3.1)(25) and the mediation package(24).

RESULTS

The PESA participants included in these analyses (n=3987) had a mean age 45.7 (4.2) y, and 62.4% were men. In total, 9.9% of participants had MS, 8.2% had insulin resistance, 44.3% were overweight, and 14.0% were obese (Table 1). The mean ox-LDL concentration was 51.8 (17.0) U/L. Almost half the individuals with MS were insulin resistant, compared with less than 5% of those who were classified as non-MS (Table 1).

After adjusting for age, sex, smoking, and LDL-cholesterol, ox-LDL was associated with higher BMI and waist circumference, triglycerides, total cholesterol, blood pressure, insulin, HOMA-IR, and HbA1c, and with lower HDL-cholesterol. Moreover, these associations remained significant after additionally adjusting for HOMA-IR, BMI, and waist circumference (Table 2). After adjustment for waist circumference, there was no positive association between ox-LDL and BMI (Table 2 and Supplementary Table 1, models 4 and 6). Similarly, after adjusting for the anthropometric variables, the association between ox-LDL and HOMA-IR substantially decreased, even becoming non-significant (Table 2 and Supplementary Table 1, models 3, 4 and 7).

The frequency of MS and its components increased across ox-LDL quartiles. With the exception of high fasting glucose, associations with MS components were independent of

HOMA-IR and anthropometric measurements (Table 3, full model). The MS component with the strongest association was high triglycerides concentration. Ox-LDL was significantly associated with insulin resistance independently of BMI and waist circumference. The ORs (95% confidence interval) for MS in the 2nd, 3rd, and 4th ox-LDL quartile versus the 1st were 0.84 (0.52, 1.36), 1.47 (0.95, 2.32), and 2.57 (1.66, 4.04) independently of HOMA-IR, BMI, and waist circumference (Table 3, full model, $p<0.001$ for trend). The 2nd, 3rd, and 4th versus 1st ox-LDL quartile ORs for the cluster of three non-anthropometric MS components were 1.52 (0.76, 3.13), 2.55 (1.36, 4.99), and 5.27 (2.88, 10.20) also independently of insulin sensitivity and anthropometry (Table 3, full model, $p<0.001$ for trend).

Analysis of ox-LDL mediation on the association between waist circumference and MS- related variables showed that ox-LDL mediated 13.9 % of the association between waist circumference and triglycerides concentration and from 1% to 3% of the association of waist circumference with HDL-cholesterol, blood pressure, and insulin (Table 4).

HOMA-IR did not mediate any of the associations between ox-LDL and MS (Supplementary Table 2); this finding is consistent with the lack of association between ox-LDL and HOMA-IR after adjustment for anthropometric variables. In contrast, most of the associations of waist circumference with the MS components were partly mediated by increased HOMA-IR, particularly for triglycerides and blood pressure (Supplementary Table 3). We also observed that higher LDL-cholesterol was only weakly associated with MS and that the association disappeared, and even reversed, once adjusted for ox-LDL (Supplementary Table 4). As a sensitivity analysis, we further adjusted the estimations of the association of ox-LDL with each MS criterion for the rest of MS criteria, and these associations still held for high waist circumference, high triglycerides, and high blood pressure (Supplementary Table 5).

DISCUSSION

In this study of 3987 non-diabetic PESA participants, ox-LDL was strongly associated with MS and its components independently of central obesity and insulin resistance. In spite of the association between ox-LDL and waist circumference, the relation between central obesity and MS components was not substantially mediated by ox-LDL. Additionally, despite it was proposed that oxidative stress may act as a cause of insulin resistance(26–28), our observations suggest that the association of ox-LDL with MS is not mediated by insulin resistance. Our analysis shows that ox-LDL variation, presumably caused by factors other than central obesity, is associated with changes in metabolic parameters. Thus, our findings suggest that ox-LDL could be a useful early predictive marker of cardiometabolic abnormalities before the appearance of insulin resistance.

Several studies have described an association between ox-LDL and MS. Holvoet et al.(29) reported that elderly individuals with MS were more likely to have high circulating levels of ox-LDL. Also, Lapointe et al.(30) noted that higher ox-LDL concentrations were associated with MS in postmenopausal women. Moreover, Ueba et al.(31) described that MS, defined according to Japanese criteria, was twice as likely among individuals with higher ox-LDL levels. Furthermore, in the Coronary Artery Risk Development in Young Adults study (CARDIA), a higher ox-LDL was associated with an increased incidence of MS(6). In fact, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), natural antioxidant defenses, are reduced in MS(32), probably consumed by increased oxidative stress. Animal studies show that this occurs in diet-induced MS as a consequence of NADPH oxidase over-activation(33). Therefore, oxidative stress was considered to play a role in the initiation and progression of metabolic disorders(2). Nonetheless, this role has not been previously addressed with mediation analysis methods in human studies, as our analysis does.

Interestingly, oxidative stress has also been contemplated as a consequence of chronic hyperglycemia and obesity(2,34). In these disorders, peroxidation species are believed to promote the development of MS(4,6,7,35,36), and oxidative stress was proposed as a mediator between obesity and MS(16). One mechanism for such process might be oxidative stress-induced insulin resistance, which is considered a key disorder in the progression to MS(11,37). In the intersection between obesity, clinical diabetes, and oxidative stress it is difficult to determine which of their pathways acts first(2). In our sample of non-diabetic individuals, with a mean BMI of 26.1 kg/m^2 and only 14% of participants obese, we were able to address early steps in MS development. In this population, ox-LDL was associated with MS independently of central obesity and insulin resistance, and was additionally associated to the lipid and blood pressure MS components, as well as their clustering.

Adiposity seems to play an important role in oxidative stress(38–42). Adipose tissue is metabolically active, and expresses inflammatory cytokines; in turn, inflammation increases reactive oxygen species(43), which dysregulate adipocytokines and might thus be involved in the pathogenesis of MS, as demonstrated in animal and human studies(2,44,45). Nonetheless, in the early stages of the MS that are the focus of the present study, increased ox-LDL linked to elevated waist circumference might not be an essential intermediate pathway connecting obesity and MS. Indeed, ox-LDL only explained 15% of the association of waist circumference with triglycerides, and very small proportions of the association with other MS components.

Oxidative stress activates kinase signaling cascades that impair insulin function, through modification and modulation of the insulin receptor and the insulin receptor substrate(11,27,46). This could be considered a compensatory mechanism to protect the cells from further increasing oxidation by limiting substrate intake(26). Oxidative stress also

inhibits insulin action by triggering signals leading to adipogenesis (adipocyte hypertrophy and hyperplasia) and inflammation(47). Insulin resistance is a core driver of MS(10), providing a plausible pathway that would explain how MS may be in part a consequence of oxidative stress. However, our data show that ox-LDL is associated with the MS independently of insulin resistance, which implies that the association does not occur through insulin resistance, at least in the early stages of the MS development. Our findings thus suggest that ox-LDL is directly associated with the development of cardiometabolic risk factors and their clustering (MS), initially acting in parallel with insulin resistance.

A possible interpretation of our findings is that the main pathophysiological change triggering MS is the shift in the metabolites used to produce energy(10), leading to lipid and hemodynamic disorders, inflammation, and atherosclerosis, with oxidative stress and insulin resistance (leading to diabetes) appearing as secondary consequences. Preferential use of fatty acids in oxidative phosphorylation produces higher levels of reactive oxygen species than oxidation of carbohydrates. Fatty-acid oxidation requires a large amount of oxygen that, in conditions of relative hypoxia due to decreased blood supply, could aggravate the situation(48–51): hypoxia favors tissue damage, macrophage infiltration, and increased adipocytokine production, ultimately increasing pro-inflammatory mediators, C-reactive protein, and plasminogen activator inhibitor-1. Triglycerides carried in lipoproteins rise in contexts of energy surplus. Additionally to energy substrate shift, lipoprotein lipase and hepatic triglyceride lipase metabolize the particles to an end form of small and dense LDL, which is particularly susceptible to oxidation. Consequently, the association between ox-LDL and triglycerides, which is the strongest that we found among the MS components, may be partly due to their common participation in lipids pathways, beyond triglycerides participation in the MS. In parallel, free fatty acids, which are highly available in situations leading to MS, induce insulin resistance by inhibiting insulin-mediated glucose uptake. In this

interpretation, oxidative stress and insulin resistance would be independent markers of the metabolic shift taking place. Consequently, ox-LDL could be used as a telltale of the early stages of cardiometabolic risk, even before the appearance of insulin resistance. Ox-LDL also contributes to the development of atherosclerosis and cardiovascular diseases(52,53,16,54,55), which are associated with MS.

This study was based on a sample of well-characterized and deeply phenotyped individuals using state-of-the-art quality control procedures. A sample size of almost 4000 individuals and modern statistical methods have allowed describing some biological processes which mediate the clustering of the risk factors in MS and have raised doubt about the relevance of some previously suggested paths. Nonetheless, the study design is cross-sectional, which limits the ability to establish that the link between oxidative stress and MS is causal. In addition, ox-LDL is one of the markers of oxidative stress and studies using a different marker might show complementary aspects of the process that links obesity and MS. Besides, regressions were adjusted for the main potential confounders, but it is possible that some residual confounding still exist due to unmeasured or unknown confounders. Analyses were adjusted for HOMA-IR as a continuous variable, which reflects a range of insulin sensitivities among non-diabetic individuals. At the early stages of metabolic disorders studied in our work, HOMA-IR was significantly associated with other metabolic variables but it did not mediate nor confound the observed associations. However, among diabetic patients, HOMA-IR reaches higher values and our results should be confirmed by future research.

In conclusion, this study shows that higher ox-LDL concentrations are associated with MS and its components independently of central obesity and insulin resistance. Levels of ox-

LDL may thus reflect core mechanisms through which MS components develop and progress in parallel with insulin resistance and could be an early sign of MS development.

ACKNOWLEDGEMENTS AND FUNDING

Dr. Laclaustra was supported in part by grants PI10/00021 and PI14/00009 from the Instituto de Salud Carlos III, co-funded by European Regional Development Fund/European Social Fund, “Investing in your future”. Yamilee Hurtado-Roca was supported by FINCyT Science and Technology Program Scholarships Nº088-FINCyT-BDE-2014 under agreement 1663/OC-PE between the Republic of Peru and the Inter-American Development Bank. The PESA study is supported by a noncompetitive unrestricted grant shared between the Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC) and Santander Bank. The CNIC is supported by the Spanish Ministry of Economy and Competitiveness (MINECO) and the Pro-CNIC Foundation, and is a Severo Ochoa Center of Excellence (MINECO award SEV-2015-0505). The authors thank Simon Bartlett (CNIC) for English editing.

DUALITY OF INTEREST

The authors declare that they have no conflict of interest in relation to this study.

AUTHOR CONTRIBUTIONS

YHR and ML drafted the manuscript; YHR and ML performed statistical analysis; HB, AFO, JMO, BI, VF, and FRA reviewed the manuscript for important intellectual content; ML, designed, and supervised this analysis. ML, AFO, JMO, BI, and VF collected data for the PESA study. VF is the principal investigator of the PESA study. All authors approved the final version. ML is the guarantor of this work and, as such, had full access to all the data in

the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem. 2006 Apr;52(4):601–23.

2. Rani V, Deep G, Singh RK, Palle K, Yadav UCS. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. Life Sci. 2016 Feb 3;

3. Trpkovic A, Resanovic I, Stanimirovic J, Radak D, Mousa SA, Cenic-Milosevic D, et al. Oxidized low-density lipoprotein as a biomarker of cardiovascular diseases. Crit Rev Clin Lab Sci. 2015 Apr;52(2):70–85.

4. Holvoet P. Obesity, the metabolic syndrome, and oxidized LDL. Am J Clin Nutr. 2006 Jun 1;83(6):1438–1438.

5. Barbosa KBF, Volp ACP, Hermsdorff HHM, Navarro-Blasco I, Zulet MÁ, Martínez JA, et al. Relationship of oxidized low density lipoprotein with lipid profile and oxidative stress markers in healthy young adults: a translational study. Lipids Health Dis. 2011;10:61.

6. Holvoet P, Lee D-H, Steffes M, Gross M, Jacobs DR. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. JAMA. 2008 May 21;299(19):2287–93.

7. Roberts CK, Sindhu KK. Oxidative stress and metabolic syndrome. Life Sci. 2009 May 22;84(21–22):705–12.

8. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res. 2002 Sep;43(9):1363–79.

9. Linna MS, Ahotupa M, Kukkonen-Harjula K, Fogelholm M, Vasankari TJ. Co-existence of insulin resistance and high concentrations of circulating oxidized LDL lipids. Ann Med. 2015 Aug 5;1–5.

10. Laclaustra M, Corella D, Ordovas JM. Metabolic syndrome pathophysiology: the role of adipose tissue. Nutr Metab Cardiovasc Dis NMCD. 2007 Feb;17(2):125–39.

11. Evans JL, Maddux BA, Goldfine ID. The molecular basis for oxidative stress-induced insulin resistance. Antioxid Redox Signal. 2005 Aug;7(7–8):1040–52.

12. Chen NG, Azhar S, Abbasi F, Carantoni M, Reaven GM. The relationship between plasma glucose and insulin responses to oral glucose, LDL oxidation, and soluble intercellular adhesion molecule-1 in healthy volunteers. Atherosclerosis. 2000 Sep;152(1):203–8.

13. Kawamura M, Heinecke JW, Chait A. Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide-dependent pathway. J Clin Invest. 1994 Aug;94(2):771–8.

14. Liguori A, Abete P, Hayden JM, Cacciatore F, Rengo F, Ambrosio G, et al. Effect of glycaemic control and age on low-density lipoprotein susceptibility to oxidation in diabetes mellitus type 1. Eur Heart J. 2001 Nov 1;22(22):2075–84.

15. Weinbrenner T, Schröder H, Escurriol V, Fito M, Elosua R, Vila J, et al. Circulating oxidized LDL is associated with increased waist circumference independent of body mass index in men and women. Am J Clin Nutr. 2006 Jan;83(1):30-35-182.

16. Matsuda M, Shimomura I. Increased oxidative stress in obesity: Implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. Obes Res Clin Pract. 2013 Oct;7(5):E330–41.

17. Fernández-Ortiz A, Jiménez-Borreguero LJ, Peñalvo JL, Ordovás JM, Mocoroa A, Fernández-Friera L, et al. The Progression and Early detection of Subclinical Atherosclerosis (PESA) study: rationale and design. Am Heart J. 2013 Dec;166(6):990–8.

18. Fernández-Friera L, Peñalvo JL, Fernández-Ortiz A, Ibañez B, López-Melgar B, Laclaustra M, et al. Prevalence, Vascular Distribution, and Multiterritorial Extent of Subclinical Atherosclerosis in a Middle-Aged Cohort: The PESA (Progression of Early Subclinical Atherosclerosis) Study. Circulation. 2015 Jun 16;131(24):2104–13.

19. World Health Organization. World Health Organization Obesity and overweight. [Internet]. WHO. 2015 [cited 2015 Oct 8]. Available from: http://www.who.int/mediacentre/factsheets/fs311/en/

20. El Assaad MA, Topouchian JA, Darné BM, Asmar RG. Validation of the Omron HEM-907 device for blood pressure measurement. Blood Press Monit. 2002 Aug;7(4):237–41.

21. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009 Oct 20;120(16):1640–5.

22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985 Jul;28(7):412–9.

23. Ascaso JF, Pardo S, Real JT, Lorente RI, Priego A, Carmena R. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. Diabetes Care. 2003 Dec;26(12):3320–5.

24. Tingley, D., Yamamoto, T., Hirose, K., Imai, K. and Keele, L. mediation: R Package for Causal Mediation Analysis. J Stat Softw [Internet]. 2014;Vol. 59, No. 5, pp. 1–38. Available from: https://www.jstatsoft.org/article/view/v059i05

25. R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.r-project.org/

26. Ceriello A. Is Oxidative Stress the Pathogenic Mechanism Underlying Insulin Resistance, Diabetes, and Cardiovascular Disease? The Common Soil Hypothesis Revisited. Arterioscler Thromb Vasc Biol. 2004 May 1;24(5):816–23.

27. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev. 2002 Oct;23(5):599–622.

28. Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J Diabetes. 2015 Apr 15;6(3):456–80.

29. Holvoet P, Kritchevsky SB, Tracy RP, Mertens A, Rubin SM, Butler J, et al. The Metabolic Syndrome, Circulating Oxidized LDL, and Risk of Myocardial Infarction in Well-Functioning

Elderly People in the Health, Aging, and Body Composition Cohort. Diabetes. 2004 Apr 1;53(4):1068–73.

30. Lapointe A, Couillard C, Piché M-È, Weisnagel SJ, Bergeron J, Nadeau A, et al. Circulating oxidized LDL is associated with parameters of the metabolic syndrome in postmenopausal women. Atherosclerosis. 2007 Abril;191(2):362–8.

31. Ueba T, Nomura S, Nishikawa T, Kajiwara M, Yamashita K. Circulating oxidized LDL, measured with FOH1a/DLH3 antibody, is associated with metabolic syndrome and the coronary heart disease risk score in healthy Japanese. Atherosclerosis. 2009 Mar;203(1):243–8.

32. Abdilla N, Tormo MC, Fabia MJ, Chaves FJ, Saez G, Redon J. Impact of the components of metabolic syndrome on oxidative stress and enzymatic antioxidant activity in essential hypertension. J Hum Hypertens. 2007 Jan;21(1):68–75.

33. Roberts CK, Barnard RJ, Sindhu RK, Jurczak M, Ehdaie A, Vaziri ND. Oxidative stress and dysregulation of NAD(P)H oxidase and antioxidant enzymes in diet-induced metabolic syndrome. Metabolism. 2006 Jul;55(7):928–34.

34. Ceriello A, Russo P dello, Amstad P, Cerutti P. High Glucose Induces Antioxidant Enzymes in Human Endothelial Cells in Culture: Evidence Linking Hyperglycemia and Oxidative Stress. Diabetes. 1996 Apr 1;45(4):471–7.

35. Kotani K, Satoh N, Kato Y, Araki R, Koyama K, Okajima T, et al. A novel oxidized low-density lipoprotein marker, serum amyloid A-LDL, is associated with obesity and the metabolic syndrome. Atherosclerosis. 2009 Jun;204(2):526–31.

36. Njajou OT, Kanaya AM, Holvoet P, Connelly S, Strotmeyer ES, Harris TB, et al. Association between oxidized LDL, obesity and type 2 diabetes in a population-based cohort, the Health, Aging and Body Composition Study. Diabetes Metab Res Rev. 2009 Nov;25(8):733–9.

37. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative Stress and Stress-Activated Signaling Pathways: A Unifying Hypothesis of Type 2 Diabetes. Endocr Rev. 2002 Oct 1;23(5):599–622.

38. Castro JP, Grune T, Speckmann B. The two faces of ROS in adipocyte function and dysfunction. Biol Chem. 2016 Mar 31;

39. Boyer F, Vidot JB, Dubourg AG, Rondeau P, Essop MF, Bourdon E. Oxidative stress and adipocyte biology: focus on the role of AGEs. Oxid Med Cell Longev. 2015;2015:534873.

40. Netzer N, Gatterer H, Faulhaber M, Burtscher M, Pramsohler S, Pesta D. Hypoxia, Oxidative Stress and Fat. Biomolecules. 2015;5(2):1143–50.

41. Manna P, Jain SK. Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. Metab Syndr Relat Disord. 2015 Dec;13(10):423–44.

42. Matusik P, Prokopowicz Z, Norek B, Olszanecka-Glinianowicz M, Chudek J, Malecka-Tendera E. Oxidative/Antioxidative status in obese and sport trained children: a comparative study. BioMed Res Int. 2015;2015:315747.

43. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. Int J Obes 2005. 2006 Mar;30(3):400–18.

44. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest. 2004 Dec;114(12):1752–61.

45. Srikanthan K, Feyh A, Visweshwar H, Shapiro JI, Sodhi K. Systematic Review of Metabolic Syndrome Biomarkers: A Panel for Early Detection, Management, and Risk Stratification in the West Virginian Population. Int J Med Sci. 2016;13(1):25–38.

46. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? Diabetes. 2003 Jan;52(1):1–8.

47. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol. 2011;29:415–45.

48. Solaini G, Baracca A, Lenaz G, Sgarbi G. Hypoxia and mitochondrial oxidative metabolism. Biochim Biophys Acta BBA - Bioenerg. 2010 Jun;1797(6–7):1171–7.

49. Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, et al. Reactive Oxygen Species Generated at Mitochondrial Complex III Stabilize Hypoxia-inducible Factor-1α during Hypoxia A MECHANISM OF O2 SENSING. J Biol Chem. 2000 Aug 18;275(33):25130–8.

50. Abramov AY, Scorziello A, Duchen MR. Three Distinct Mechanisms Generate Oxygen Free Radicals in Neurons and Contribute to Cell Death during Anoxia and Reoxygenation. J Neurosci. 2007 Jan 31;27(5):1129–38.

51. Dugan LL, Choi DW. Free Radicals in Hypoxia-Ischemia. 1999 [cited 2016 Apr 1]; Available from: http://www.ncbi.nlm.nih.gov/books/NBK28241/

52. Husain K, Hernandez W, Ansari RA, Ferder L. Inflammation, oxidative stress and renin angiotensin system in atherosclerosis. World J Biol Chem. 2015 Aug 26;6(3):209–17.

53. Maiolino G, Rossitto G, Caielli P, Bisogni V, Rossi GP, Calò LA. The role of oxidized low-density lipoproteins in atherosclerosis: the myths and the facts. Mediators Inflamm. 2013;2013:714653.

54. Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J Diabetes. 2015 Apr 15;6(3):456–80.

55. Kawada T. Oxidative stress markers and cardiovascular disease: advantage of using these factors in combination with lifestyle factors for cardiovascular risk assessment. Int J Cardiol. 2012 May 17;157(1):119–20.

TABLES

Table 1. Characteristics of study participants.

	Total N=3987	**With metabolic syndrome** N=393 9.9%	**Without metabolic syndrome** N=3594 90.1%	**p value**
Male gender	62.4 [2488]	85.5 [336]	59.9 [2152]	<0.001
Age, y	45.7 (4.2)	47.6 (4.1)	45.5 (4.2)	<0.001
BMI, kg/m^2	26.1 (3.8)	30.9 (3.5)	25.5 (3.4)	<0.001
Waist circumference, cm	89.1 (11.9)	104.5 (9.3)	87.4 (10.9)	<0.001
Triglycerides, mg/dL	93.4 (54.5)	157.4 (79.7)	86.4 (45.9)	<0.001
HDL-c, mg/dL	49.2 (12.2)	38.5 (7.3)	50.4 (12.0)	<0.001
Total cholesterol, mg/dL	200.7 (33.0)	209.8 (36.1)	199.7 (32.5)	<0.001
LDL-c, mg/dL	132.6 (29.6)	139.6 (31.4)	131.9 (29.3)	<0.001
Systolic blood pressure, mmHg	116.0 (12.4)	129.1 (13.0)	114.6 (11.5)	<0.001
Diastolic blood pressure, mmHg	72.4 (9.4)	82.7 (9.4)	71.2 (8.7)	<0.001
Fasting glucose, mg/dL	89.4 (8.7)	99.7 (9.1)	88.3 (7.9)	<0.001
Insulin, pmol/L	5.9 (3.5)	10.9 (4.9)	5.4 (2.9)	<0.001
HOMA-IR	1.3 (0.9)	2.7 (1.3)	1.2 (0.7)	<0.001
Hemoglobin A1c, %	5.4 (0.4)	5.6 (0.4)	5.4 (0.4)	<0.001
Ox-LDL, U/L	51.8 (17.0)	61.4 (19.5)	50.7 (16.3)	<0.001
Insulin resistance	8.2 [328]	44.5 [175]	4.3 [153]	<0.001
Obesity	14.0 [560]	56.2 [221]	9.4 [339]	<0.001
Overweight	44.3 [1765]	39.4 [155]	44.8 [1610]	0.048
Smoking	28.2 [1123]	30.5 [120]	27.9 [1003]	0.298

Data are presented as mean (standard deviation) or percentage [count].
BMI: Body Mass Index
HDL-c: High-Density Lipoprotein Cholesterol
LDL-c: Low-Density Lipoprotein Cholesterol
HOMA-IR: Homeostatic Model Assessment—Insulin Resistance.
Ox-LDL: Oxidized Low-Density Lipoprotein
Mean hemoglobin A1c was 36 mmol/mol for the whole sample and those without metabolic syndrome, and 38 mmol/mol for those with metabolic syndrome; all standard deviations were 4.4 mmol/mol.

Table 2. Mean values and adjusted differences (95% confidence interval) in metabolic syndrome-related parameters for comparison of the three highest ox-LDL quartiles with the first quartile

	Quartiles of ox-LDL, U/L				
	[lowest,39.9]	**(39.9,49.4]**	**(49.4,60.8]**	**(60.8,highest]**	**p trend**
N	997	997	996	997	
Mean ox-LDL, U/L	33.06	44.72	54.69	74.64	
Body mass index, kg/m^2	**24.93**	**25.58**	**26.41**	**27.37**	
Basic model	0.00	0.23	0.61	1.03	<0.001
	(Reference)	(-0.07,0.53)	(0.29,0.93)	(0.68,1.39)	
Full model *	0.00	-0.02	-0.04	-0.20	0.02
	(Reference)	(-0.17,0.14)	(-0.20,0.13)	(-0.38,-0.02)	
Waist circumference, cm	**84.72**	**87.15**	**90.20**	**94.20**	
Basic model	0.00	0.86	2.12	3.91	<0.001
	(Reference)	(0.04,1.68)	(1.25,2.99)	(2.94,4.87)	
Full model *	0.00	0.42	0.76	1.43	<0.001
	(Reference)	(0.01,0.82)	(0.33,1.19)	(0.95,1.91)	
Triglycerides, mg/dL	**71.87**	**82.64**	**95.22**	**123.97**	
Basic model	0.00	8.41	17.48	42.47	<0.001
	(Reference)	(4.06,12.76)	(12.87,22.08)	(37.36,47.59)	
Full model	0.00	8.99	16.47	38.20	<0.001
	(Reference)	(4.91,13.07)	(12.14,20.80)	(33.37,43.03)	
HDL-cholesterol, mg/dL	**52.17**	**50.46**	**48.74**	**45.56**	
Basic model	0.00	-1.08	-1.78	-3.75	<0.001
	(Reference)	(-2.04,-0.13)	(-2.80,-0.77)	(-4.88,-2.63)	
Full model	0.00	-1.04	-1.31	-2.55	<0.001
	(Reference)	(-1.94,-0.13)	(-2.28,-0.35)	(-3.62,-1.47)	
Total cholesterol, mg/dL	**178.75**	**191.46**	**206.37**	**226.16**	
Basic model	0.00	0.59	1.71	4.75	<0.001
	(Reference)	(-0.51,1.70)	(0.54,2.89)	(3.44,6.05)	
Full model	0.00	0.76	1.98	5.10	<0.001
	(Reference)	(-0.35,1.86)	(0.80,3.15)	(3.79,6.41)	
LDL-cholesterol, mg/dL	**112.05**	**124.34**	**138.44**	**155.65**	
Basic model *	0.00	11.64	25.18	41.83	<0.001
	(Reference)	(9.48,13.81)	(22.99,27.37)	(39.59,44.06)	
Full model *	0.00	11.65	24.84	40.93	<0.001
	(Reference)	(9.49,13.81)	(22.65,27.03)	(38.67,43.19)	
Systolic blood pressure, mmHg	**112.80**	**114.50**	**117.16**	**119.72**	
Basic model	0.00	0.40	1.72	2.57	<0.001
	(Reference)	(-0.56,1.36)	(0.70,2.74)	(1.45,3.70)	
Full model	0.00	0.27	1.20	1.50	<0.001
	(Reference)	(-0.64,1.19)	(0.23,2.17)	(0.41,2.58)	
Diastolic blood pressure, mmHg	**69.95**	**71.25**	**73.15**	**75.09**	
Basic model	0.00	0.37	1.39	2.27	<0.001
	(Reference)	(-0.40,1.15)	(0.57,2.21)	(1.36,3.18)	
Full model	0.00	0.22	0.81	1.08	0.001
	(Reference)	(-0.49,0.93)	(0.05,1.57)	(0.23,1.92)	
Fasting glucose, mg/dL	**87.94**	**88.47**	**90.10**	**91.28**	
Basic model	0.00	-0.42	0.22	0.25	0.06
	(Reference)	(-1.13,0.29)	(-0.52,0.97)	(-0.58,1.08)	
Full model	0.00	-0.23	0.06	-0.65	0.27
	(Reference)	(-0.84,0.37)	(-0.58,0.71)	(-1.37,0.06)	
Insulin, pmol/L	**5.26**	**5.39**	**5.99**	**7.15**	
Basic model	0.00	-0.14	0.18	1.00	<0.001
	(Reference)	(-0.44,0.17)	(-0.14,0.50)	(0.64,1.35)	
Full model	0.00	0.00	0.06	0.31	<0.001
	(Reference)	(-0.12,0.13)	(-0.07,0.19)	(0.16,0.46)	
HOMA-IR, log	**-0.01**	**0.02**	**0.13**	**0.31**	
Basic model	0.00	-0.03	0.01	0.12	<0.001
	(Reference)	(-0.08,0.02)	(-0.04,0.07)	(0.06,0.18)	
Full model *	0.00	-0.06	-0.05	0.00	0.21
	(Reference)	(-0.10,-0.01)	(-0.10,-0.01)	(-0.06,0.05)	
Hemoglobin A1c, %	**5.31**	**5.38**	**5.44**	**5.46**	
Basic model	0.00	0.04	0.07	0.07	0.007
	(Reference)	(0.01,0.07)	(0.04,0.10)	(0.03,0.10)	
Full model	0.00	0.05	0.07	0.06	0.04
	(Reference)	(0.01,0.08)	(0.04,0.10)	(0.02,0.09)	

Bold lines are unadjusted means. Differences are estimated from linear regression models with adjustment for age, sex, smoking status, and LDL-cholesterol (basic model), and additionally for HOMA-IR (log), body mass index, and waist circumference (full model).
* In these regressions the outcome variable was excluded from the adjustment variables.
Mean hemoglobin A1c were 34.6, 35.2, 35.9, and 36.1 mmol/mol for quartiles 1st to 4th of ox-LDL respectively. The differences expressed in mmol/mol were approximately 10 times the figures in the table.

Table 3. Percentages and adjusted odds ratios (95% confidence interval) for metabolic syndrome and its related components for comparison of the three highest ox-LDL quartiles with the first quartile

	Quartiles of ox-LDL, U/L				
	[lowest,39.9]	**(39.9,49.4]**	**(49.4,60.8]**	**(60.8,highest]**	**p trend**
N	997	997	996	997	
Average ox-LDL	33.06	44.72	54.69	74.64	
High waist circumference	**13.7**	**17.4**	**21.4**	**30.2**	
Basic model	1.00	1.21	1.48	2.24	<0.001
	(Reference)	(0.95,1.55)	(1.15,1.90)	(1.71,2.93)	
Full model *	1.00	1.04	1.23	1.70	0.008
	(Reference)	(0.71,1.54)	(0.84,1.81)	(1.13,2.55)	
High triglycerides	**3.7**	**5.5**	**9.0**	**24.5**	
Basic model	1.00	1.39	2.12	6.38	<0.001
	(Reference)	(0.90,2.16)	(1.41,3.23)	(4.30,9.66)	
Full model	1.00	1.52	2.08	5.83	<0.001
	(Reference)	(0.98,2.40)	(1.37,3.21)	(3.89,8.92)	
Low HDL-cholesterol	**26.9**	**31.3**	**31.9**	**39.6**	
Basic model	1.00	1.28	1.33	1.94	<0.001
	(Reference)	(1.05,1.56)	(1.08,1.64)	(1.55,2.44)	
Full model	1.00	1.30	1.26	1.66	0.001
	(Reference)	(1.06,1.60)	(1.01,1.56)	(1.31,2.11)	
High blood pressure	**13.4**	**14.7**	**21.8**	**27.9**	
Basic model	1.00	0.95	1.44	1.82	<0.001
	(Reference)	(0.73,1.24)	(1.11,1.87)	(1.38,2.41)	
Full model	1.00	0.93	1.33	1.47	0.002
	(Reference)	(0.71,1.23)	(1.02,1.75)	(1.10,1.96)	
High fasting glucose	**8.3**	**9.2**	**12.7**	**17.5**	
Basic model	1.00	0.89	1.06	1.27	0.006
	(Reference)	(0.64,1.23)	(0.77,1.46)	(0.91,1.77)	
Full model	1.00	0.95	0.97	0.90	0.88
	(Reference)	(0.67,1.35)	(0.69,1.38)	(0.63,1.29)	
Metabolic syndrome	**5.4**	**5.1**	**9.8**	**19.1**	
Basic model	1.00	0.83	1.61	3.36	<0.001
	(Reference)	(0.56,1.25)	(1.12,2.34)	(2.33,4.91)	
Full model	1.00	0.84	1.47	2.57	<0.001
	(Reference)	(0.52,1.36)	(0.95,2.32)	(1.66,4.04)	
Insulin resistance	**4.7**	**5.2**	**8.1**	**14.8**	
Basic model	1.00	0.95	1.36	2.32	<0.001
	(Reference)	(0.63,1.44)	(0.92,2.02)	(1.57,3.47)	
Full model *	1.00	0.89	1.10	1.65	<0.001
	(Reference)	(0.56,1.39)	(0.72,1.70)	(1.08,2.56)	
2-or-more non-waist criteria	**10.3**	**11.1**	**18.6**	**31.2**	
Basic model	1.00	0.95	1.59	2.95	<0.001
	(Reference)	(0.71,1.27)	(1.20,2.11)	(2.21,3.96)	
Full model	1.00	0.97	1.51	2.42	<0.001
	(Reference)	(0.70,1.34)	(1.11,2.07)	(1.76,3.34)	
3-or-more non-waist criteria	**1.6**	**2.2**	**4.4**	**10.5**	
Basic model	1.00	1.26	2.51	6.32	<0.001
	(Reference)	(0.65,2.47)	(1.39,4.73)	(3.57,11.82)	
Full model	1.00	1.52	2.55	5.27	<0.001
	(Reference)	(0.76,3.13)	(1.36,4.99)	(2.88,10.20)	

Bold lines are unadjusted proportions. Odd ratios are estimated from logistic regression models with adjustment for age, sex, smoking status, and LDL-cholesterol (basic model), and additionally for HOMA-IR (log), body mass index and waist circumference (full model).
* In these regressions the variable used in the direct definition of the outcome variable (waist circumference or HOMA-IR) was excluded from the adjustment variables.

Table 4. Ox-LDL-mediated fraction of the effect of waist circumference on metabolic syndrome-related parameters

Outcome (per cm of waist)	Total	Direct	Mediated	Mediated %
Body mass index, kg/m^2	0.3163 (0.3094,0.3234) p<0.001	0.3172 (0.3104,0.3243) p<0.001	-0.0009 (-0.0017,-0.0001) p=0.02	-
Triglycerides, mg/dL	1.3557 (1.1851,1.5279) p<0.001	1.1671 (0.9944,1.3415) p<0.001	0.1886 (0.1358,0.2497) p<0.001	13.9% (10.1,18.5)% p<0.001
HDL-cholesterol, mg/dL	-0.3364 (-0.3714,-0.3023) p<0.001	-0.3276 (-0.3623,-0.2921) p<0.001	-0.0088 (-0.0139,-0.0042) p<0.001	2.6% (1.2,4.2)% p<0.001
Total cholesterol, mg/dL	-0.0648 (-0.1042,-0.0210) p=0.006	-0.0938 (-0.1334,-0.0480) p<0.001	0.0290 (0.0193,0.0394) p<0.001	-
Systolic BP, mmHg	0.3233 (0.2876,0.3595) p<0.001	0.3143 (0.2771,0.3515) p<0.001	0.0091 (0.0041,0.0147) p<0.001	2.8% (1.3,4.6)% p<0.001
Diastolic BP, mmHg	0.3410 (0.3122,0.3702) p<0.001	0.3352 (0.3058,0.3657) p<0.001	0.0058 (0.0020,0.0095) p<0.001	1.7% (0.6,2.8)% p<0.001
Fasting glucose, mg/dL	0.2540 (0.2281,0.2788) p<0.001	0.2551 (0.2286,0.2798) p<0.001	-0.0011 (-0.0047,0.0025) p=0.52	-
Insulin, pmol/L	0.1946 (0.1834,0.2075) p<0.001	0.1923 (0.1808,0.2051) p<0.001	0.0023 (0.0007,0.0040) p=0.008	1.2% (0.4,2.1)% p=0.008
HOMA-IR, log	0.0329 (0.0312,0.0346) p<0.001	0.0328 (0.0311,0.0344) p<0.001	0.0001 (-0.0001,0.0003) p=0.29	0.3% (-0.3,1.0)% p=0.29
Hemoglobin A1c, %	0.0031 (0.0018,0.0042) p<0.001	0.0029 (0.0016,0.0041) p<0.001	0.0002 (0.0000,0.0003) p=0.05	5.3% (-0.1,13.7)% p=0.05

BP: Blood pressure.

The columns show the total effect, direct effect, and ox-LDL-mediated effect of waist circumference on each metabolic syndrome-related parameter, and the proportion of the total effect of waist circumference that is mediated by ox-LDL. Figures in parentheses show the 95% confidence interval calculated by nonparametric bootstrapping. The basic adjustment model was used (age, sex, smoking status, and LDL-cholesterol).