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Oxidized LDL Is Associated With Metabolic Syndrome Traits Independently of Central Obesity and Insulin Resistance

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This study assesses whether oxidative stress, using oxidized LDL (ox-LDL) as a 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 3,987 subjects without diabetes in the Progression of Early Subclinical Atherosclerosis (PESA) Study. For the second, third, and fourth ox-LDL quartiles versus the first, the odds ratios (95% CI) 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, BMI, waist circumference, and HOMA-insulin resistance (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. This study found 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.

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 LDL (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 the elevation of small and dense LDL particles, which are easily oxidized (8). High ox-LDL levels are also associated with insulin resistance (9), which is tightly linked to the pathogenesis of MS (10). Insulin resistance may arise from the oxidative stress–mediated activation of kinase signaling cascades that phosphorylate insulin receptors, leading to impaired insulin action (11), but 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 has been involved with the induction of oxidative stress

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(15), which in turn may contribute to the development of MS (16).

No previous study, however, 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 a size big enough to address subtle associations, this study assesses *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 Fig. 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. 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 4,117 participants, we excluded 82 with diabetes, 2 with missing ox-LDL data, and 46 with no recorded smoking status. Data were complete for all other relevant variables. The final analytical sample thus included 3,987 individuals.

Data Collection

Data were obtained from structured clinical interviews and questionnaires, a physical examination, 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. BMI was calculated as weight divided by height squared (kg/m^2); overweight was defined as BMI between 25 and 30 kg/m^2, and obesity as BMI $\geq$30 kg/m^2 (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 min before the measurements were made, and blood pressure readings were made at 1-min intervals. All procedures were International Organization for Standardization 9001 certified.

Laboratory Measurements

Peripheral venous blood was collected after an 8-h fast. A monoclonal antibody 4E6–based competition ELISA (Mercodia AB, Uppsala, Sweden) was used for measuring plasma levels of ox-LDL. Monoclonal antibody 4E6 is directed against a conformational epitope in the apolipoprotein B-100 moiety of LDL that is generated as a consequence of substitution of lysine residues of apolipoprotein B-100 with aldehydes. Whole-blood HbA_{1c} was measured by reverse-phase cationic exchange chromatography and double wavelength colorimetric quantification (D-10 Hemoglobin Testing System, Bio-Rad). Triglycerides, total cholesterol, HDL-cholesterol, and glucose were measured in serum with spectrophotometric assays in the Architect-Ci8200 analyzer (Instrumentation Laboratory) using the manufacturer's kits. Insulin was determined in the same analyzer by chemiluminescence immunoassay. LDL-cholesterol was calculated from the Friedewald equation.

MS and Insulin Resistance

According to the 2009 harmonizing definition (21), MS was diagnosed when participants met at least three of the following five criteria: high waist circumference ($\geq$102 cm in men and $\geq$88 cm in women), high triglycerides ($\geq$1.7 mmol/L [$\geq$150 mg/dL]), low HDL-cholesterol ($<$1.0 mmol/L [$<$40 mg/dL] in men and $<$1.3 mmol/L [$<$50 mg/dL] in women), high blood pressure ($\geq$130/85 mmHg or treatment with antihypertensive medication), and high fasting glucose ($\geq$5.6 mmol/L [$\geq$100 mg/dL] or drug treatment for elevated glucose).

HOMA-insulin resistance (HOMA-IR) was calculated as glucose (mg/dL) multiplied by insulin (μU/mL) and divided by 405 (22). Insulin resistance was defined as HOMA-IR $\geq$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% 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 included 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.

Because 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 nonanthropometric MS components: two or more or three or more 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 nonparametric bootstrapping with 1,000 resamples and percentile-based confidence intervals (24).

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

RESULTS

The 3,987 PESA participants (62.4% men) included in these analyses were of a mean (standard deviation) age of 45.7 (4.2) years, and 9.9% had MS, 8.2% had insulin resistance, 44.3% were overweight, and 14.0% were obese (Table 1). The mean (standard deviation) ox-LDL concentration was 51.8 (17.0) units/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 adjustments were made 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 HbA_{1c} and with lower HDL-cholesterol. These associations also 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, the association between ox-LDL and HOMA-IR substantially decreased after adjusting for the anthropometric variables, even becoming nonsignificant (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% CI) for MS in the second, third, and fourth ox-LDL quartile versus the first were 0.84 (0.52, 1.36), 1.47 (0.95, 2.32), and 2.57 (1.66, 4.04), respectively, independently of HOMA-IR, BMI, and waist circumference ($P < 0.001$ for trend) (Table 3, full model). The second, third, and fourth versus the first ox-LDL quartile ORs for the cluster of three nonanthropometric MS components were 1.52 (0.76, 3.13), 2.55 (1.36, 4.99), and 5.27 (2.88, 10.20), respectively, also independently of insulin sensitivity and anthropometry ($P < 0.001$ for trend) (Table 3, full model).

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 1–3% of the association of waist circumference with HDL-cholesterol, blood pressure, and insulin (Table 4).

Table 1—Characteristics of study participants

	Total N = 3,987	With metabolic syndrome n = 393 (9.9%)	Without metabolic syndrome n = 3,594 (90.1%)	P value
Male sex	62.4 [2,488]	85.5 [336]	59.9 [2,152]	<0.001
Age, years	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-cholesterol, 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-cholesterol, 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
HbA_{1c}, %	5.4 (0.4)	5.6 (0.4)	5.4 (0.4)	<0.001
HbA_{1c}, mmol/mol	36 (4.4)	38 (4.4)	36 (4.4)	<0.001
Ox-LDL, units/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 [1,765]	39.4 [155]	44.8 [1,610]	0.048
Smoking	28.2 [1,123]	30.5 [120]	27.9 [1,003]	0.298

Data are presented as mean (standard deviation) or percentage [count].

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

	Quartiles of ox-LDL, units/L				
	≤39.9 *n* = 997	>39.9 and ≤49.4 *n* = 997	>49.4 and ≤60.8 *n* = 996	>60.8 *n* = 997	*P* for trend
Mean ox-LDL, units/L	33.06	44.72	54.69	74.64	
BMI, 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)	

Continued on p. 478

Table 2—Continued

	Quartiles of ox-LDL, units/L				
	≤39.9 *n* = 997	>39.9 and ≤49.4 *n* = 997	>49.4 and ≤60.8 *n* = 996	>60.8 *n* = 997	*P* for trend
HbA_{1c}, %†	**5.31**	**5.38**	**5.44**	**5.46**	
Basic model	0.00 (Reference)	0.04 (0.01, 0.07)	0.07 (0.04, 0.10)	0.07 (0.03, 0.10)	0.007
Full model	0.00 (Reference)	0.05 (0.01, 0.08)	0.07 (0.04, 0.10)	0.06 (0.02, 0.09)	0.04

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), BMI, and waist circumference (full model). Unadjusted means appear in boldface type. *The outcome variable in these regressions was excluded from the adjustment variables. †Mean HbA_{1c} values were 34.6, 35.2, 35.9, and 36.1 mmol/mol for first to fourth quartiles of ox-LDL, respectively. The differences expressed in mmol/mol were approximately 10 times the data in the table.

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

This study of 3,987 PESA participants without diabetes found ox-LDL was strongly associated with MS and its components independently of central obesity and insulin resistance. Despite the association between ox-LDL and waist circumference, the relation between central obesity and MS components was not substantially mediated by ox-LDL. In addition, despite the proposal 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, Lapointe et al. (30) noted that higher ox-LDL concentrations were associated with MS in postmenopausal women, and Ueba et al. (31) described that MS, defined according to Japanese criteria, was twice as likely among individuals with higher ox-LDL levels. The Coronary Artery Risk Development in Young Adults (CARDIA) study also found a higher ox-LDL was associated with an increased incidence of MS (6). In fact, superoxide dismutase and glutathione peroxidase, 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 overactivation (33). Oxidative stress was therefore 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 a process might be oxidative stress–induced insulin resistance, which is considered a key disorder in the progression to MS (11, 27). In the intersection between obesity, clinical diabetes, and oxidative stress, determining which of their pathways acts first is difficult (2). In our sample of individuals without diabetes, 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. Ox-LDL in this population 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 (37–41). Adipose tissue is metabolically active and expresses inflammatory cytokines; in turn, inflammation increases reactive oxygen species (42), which dysregulate adipocytokines and might thus be involved in the pathogenesis of MS, as demonstrated in animal and human studies (2,43,44). Nonetheless, in the early stages of the MS that are the focus of the current 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

Table 3—Percentages and adjusted ORs (95% CI) for metabolic syndrome and its related components for comparison of the three highest ox-LDL quartiles with the first quartile

	Quartiles of ox-LDL, units/L				
	≤39.9 *n* = 997	>39.9 and ≤49.4 *n* = 997	>49.4 and ≤60.8 *n* = 996	>60.8 *n* = 997	*P* for trend
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 nonwaist 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 nonwaist 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)	

ORs are estimated from logistic regression models with adjustment for age, sex, smoking status, and LDL-cholesterol (basic model), and additionally for HOMA-IR (log), BMI, and waist circumference (full model). Unadjusted proportions appear in boldface type. *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.

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 (11,27,45). 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

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 %
BMI, 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 blood pressure, 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 blood pressure, 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) $P = 0.29$
HbA_{1c}, %	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) $P = 0.05$

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

inflammation (46). Insulin resistance is a core driver of MS (10), providing a plausible pathway that would explain how MS may partly be a consequence of oxidative stress. However, our data show that ox-LDL is associated with MS independently of insulin resistance, which implies that the association does not occur through insulin resistance, at least in the early stages of 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. Oxidation of fatty acids requires a large amount of oxygen that, in conditions of relative hypoxia caused by decreased blood supply, could aggravate the situation (47–50): hypoxia favors tissue damage, macrophage infiltration, and increased adipocytokine production, ultimately increasing proinflammatory mediators, C-reactive protein, and plasminogen activator inhibitor 1. Triglycerides carried in lipoproteins rise in contexts of energy surplus. In addition to the 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 a result of their common participation in lipids pathways, beyond triglycerides participation in 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 (16,28,51–53), 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 4,000 individuals and modern statistical methods have allowed describing some biological processes that 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 that some residual confounding still exists is possible because of unmeasured or unknown confounders. Analyses were adjusted for HOMA-IR as a continuous variable, which reflects a range of insulin sensitivities among individuals without diabetes. At the early stages of metabolic disorders studied in our work, HOMA-IR was significantly associated with other metabolic variables but did not mediate nor confound the observed associations. However, HOMA-IR reaches higher values among patients with diabetes, 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.

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