The Metabolic Syndrome, Circulating Oxidized LDL, and Risk of Myocardial Infarction in Well-Functioning Elderly People in the Health, Aging, and Body Composition Cohort

Paul Holvoet,[1] Stephen B. Kritchevsky,[2] Russell P. Tracy,[3,4] Ann Mertens,[1] Susan M. Rubin,[5] Javed Butler,[6] Bret Goodpaster,[7] and Tamara B. Harris[8]

The object of this study was to establish the association between the metabolic syndrome and oxidized LDL (oxLDL) and to determine the risk for coronary heart disease (CHD) in relation to the metabolic syndrome and levels of oxLDL. OxLDL was measured in plasma from 3,033 elderly participants in the Health, Aging, and Body Composition study. The metabolic syndrome was defined according to criteria established in the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. We observed that the metabolic syndrome was associated with higher levels of oxLDL due to a higher fraction of oxLDL, not to higher levels of LDL cholesterol. Individuals with the metabolic syndrome had twice the odds of having high oxLDL (>1.90 mg/dl) compared with those not having the metabolic syndrome, after adjusting for age, sex, ethnicity, smoking status, and LDL cholesterol. Among those participants who had the metabolic syndrome at study entry, incidence rates of future CHD events were 1.6-fold higher, after adjusting for age, sex, ethnicity, and smoking status. OxLDL was not an independent predictor of total CHD risk. However, those with high oxLDL showed a greater disposition to myocardial infarction (relative risk 2.25, 95% confidence interval 1.22–4.15). We concluded that the metabolic syndrome, a risk factor for CHD, is associated with higher levels of circulating oxLDL that are associated with a greater disposition to atherothrombotic coronary disease. *Diabetes* 53: 1068–1073, 2004

From the [1]Center for Experimental Surgery and Anesthesiology, Catholic University, Leuven, Belgium; the [2]J. Paul Sticht Center on Aging, Wake Forest University Health Sciences, Winston-Salem, North Carolina; the [3]Department of Pathology, University of Vermont, Burlington, Vermont; the [4]Department of Biochemistry, University of Vermont, Burlington, Vermont; the [5]Prevention Sciences Group, University of California, San Francisco, California; [6]Vanderbilt University, Nashville, Tennessee; the [7]Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania; and the [8]Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, National Institutes of Health, Bethesda, Maryland.

Address correspondence and reprint requests to Paul Holvoet, PhD, Center for Experimental Surgery and Anesthesiology, Katholieke Universiteit Leuven, Herestraat 49, B-3000 Leuven, Belgium. E-mail: paul.holvoet@med.kuleuven.ac.be.

Received for publication 23 June 2003 and accepted in revised form 9 January 2004.

ATP III, Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; CHD, coronary heart disease; CVD, cardiovascular disease; ELISA, enzyme-linked immunosorbent assay; Health ABC, Health, Aging, and Body Composition; MI, myocardial infarction; NHANES III, Third National Health and Nutrition Examination Survey; oxLDL, oxidized LDL.

Oxidized LDL (oxLDL) has been shown to play an important role in the pathogenesis of atherosclerosis (1–3). We and others have demonstrated an association between cardiovascular disease (CVD) and oxidation of LDL (4–6). We have also found circulating oxLDL to be a prognostic marker of CVD in cardiac transplant patients (7). In middle-aged people, obesity and dyslipidemia are the strongest predictors of levels of oxLDL (8). Recently, the association between dyslipidemia and oxidation of LDL has been demonstrated in individuals in the pre-diabetic state (9). Finally, we have shown that in the Health, Aging, and Body Composition (Health ABC) cohort a high coronary heart disease (CHD) risk status (based on Framingham score) before CHD events is associated with high levels of circulating oxLDL, even after adjustment for LDL cholesterol (10).

Individuals with the metabolic syndrome are at increased risk for developing CHD as well as for mortality from CHD and other causes (11,12). The Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III) (13) drew attention to the importance of the metabolic syndrome and provided a working definition of this syndrome. Findings from the Third National Health and Nutrition Examination Survey (NHANES III) showed that the metabolic syndrome is highly prevalent within the U.S.; that prevalence increased from 6.7% among participants ages 20–29 years to 43.5 and 42.0% for participants ages 60–69 years and ≥70 years, respectively (14).

Because the metabolic syndrome is associated with high risk for atherosclerotic disease, a process thought to involve LDL oxidation, we examined the relation between metabolic syndrome components and circulating oxLDL levels in the Health ABC cohort. Because we have found an association between the metabolic syndrome and higher prevalence of high levels of oxLDL, we sought to evaluate in a large-scale elderly population the potential relation among the metabolic syndrome, circulating oxLDL, and incident CHD events.

RESEARCH DESIGN AND METHODS

The study population was comprised of 3,033 participants in the baseline exam of the Health ABC study. Participants were enrolled between March 1997 and June 1998. The study's target population was individuals age 70–79 years who did not have lower extremity functional limitation and resided in

the Memphis, Tennessee, and Pittsburgh, Pennsylvania, areas. These criteria selected the best functioning 40–60% of the older population. The socioeconomic status patterns reflected the locations from which the samples were drawn: 56.4% of black participants vs. 87.8% of white participants had completed high school, and the median income category was $10,000–25,000 for black participants and $25,000–50,000 for white participants. A participant's well-functioning was determined by self-report and defined as having the individual having no difficulty in walking a quarter of a mile or going up 10 steps without resting reported on two separate occasions. Exclusion criteria included difficulties with daily living activities, obvious cognitive impairment, an inability to communicate with the interviewer, an intention of moving within 3 years, therapy for cancer within the prior 3 years, and previous participation in a trial involving a lifestyle intervention.

Incident CHD was defined by coronary death or any overnight hospitalization in an acute care hospital for myocardial infarction (MI), angina, coronary angioplasty or artery bypass surgery, or chronic heart failure. Events and diagnoses were adjudicated based on hospital and death records. Participants were asked to report any hospitalizations, and every 6 months they were asked direct questions to elicit information about any event. When an event was reported, hospitalization records were collected and verified by a Health ABC Disease Adjudication Committee. Date and causes of death were taken from the death certificate. Only events that were confirmed by the adjudication committee were included.

Based on cardiac procedures and diagnoses reported to the U.S. Department of Health and Human Services' Centers for Medicare and Medicaid Services in the 5 years before enrollment, 385 participants had CHD (MI, angina, coronary angioplasty or artery bypass surgery, or chronic heart failure). Based on ATP III criteria, 1,113 had CHD risk equivalents: noncoronary forms of clinical atherosclerotic disease (n = 232), diabetes (n = 577), and/or a 10-year risk for CHD events >20% by Framingham scoring (n = 1,023). Of those with CVD, 60% were treated with antianginal drugs; 42% with antiarrhythmic drugs; 35% with vasodilators; 46% with antiplatelet drugs, including aspirin; 90% with blood pressure–lowering drugs; and 21% with lipid-lowering drugs (10). Since being enrolled in the study (follow-up through June 2002), 418 participants had a CHD event (MI, angina, coronary angioplasty or artery bypass surgery, chronic heart failure, or cardiovascular death). There were 238 events in participants without baseline CHD. Among these, 120 had at least one MI: 106 had one and 14 had more than one based on electrocardiogram and enzyme changes. There were 88 primary and 32 secondary MIs.

During an in-clinic visit, fasting blood specimens were drawn and processed, with plasma and serum stored at −80°C. From these blood samples, total and HDL cholesterol and serum triglycerides were measured using a Vitros 950 analyzer (Johnson & Johnson). The interassay coefficient of variation (CV) was 1.5% for total cholesterol, 2.3% for triglycerides, and 2.3% for HDL cholesterol. LDL cholesterol levels were calculated by the Friedewald equation (15). Levels of oxLDL were measured (2000–2001) blindly at the Center for Experimental Surgery and Anesthesiology. An mAb-4E6–based competition enzyme-linked immunosorbent assay (ELISA) was used for measuring plasma oxLDL levels (5,16,17). The monoclonal antibody mAb-4E6 is directed against a conformational epitope in the apoB-100 moiety of LDL that is generated by substituting aldehydes for at least 60 lysine residues of apolipoprotein B-100. This number of substituted lysines corresponds to the minimal number of substituted lysines required for scavenger-mediated uptake of oxLDL. Substituting aldehydes can be produced by peroxidation of LDL lipids, resulting in the generation of oxLDL. Aldehydes that are released by endothelial cells under oxidative stress or by activated platelets may also induce the oxidative modification of apolipoprotein B-100 in the absence of the peroxidation of LDL lipids. Specificity of the assay is excellent, as the C_{50} values (i.e., concentrations required to obtain 50% inhibition of antibody binding in the ELISA) are 25 mg/dl for native LDL isolated by ultracentrifugation from the plasma of healthy volunteers and 0.025 mg/dl for oxLDL with at least 60 aldehyde-substituted lysines per apolipoprotein B-100 obtained by copper-ion induced oxidation of the same LDL (16). Therefore, 160 mg/dl LDL in a person's plasma would contribute <0.2 mg/dl in the oxLDL assay. The interassay CV of oxLDL is 12%.

Measurements of waist circumference were taken with participants standing and were made at the level of widest circumference in the area between the iliac crest and lower rib. Blood pressure was taken three times with participants in the seated position after 5 min of quiet rest. The average of the last two measurements was used for systolic and diastolic blood pressure.

Metabolic syndrome components were defined as detailed in the ATP III report: *1*) waist circumference >102 cm in men and >88 cm in women, *2*) fasting triglycerides ≥1.70 mmol/l (150 mg/dl), *3*) HDL cholesterol <1.03 mmol/l (40 mg/dl) in men and <1.29 mmol/l (50 mg/dl) in women, *4*) blood pressure ≥130/85 mmHg, and *5*) fasting-glucose ≥6.10 mmol/l (110 mg/dl). Participants with at least three of these components were determined to have the metabolic syndrome.

Before measuring oral glucose tolerance, participants (excluding those taking insulin or hypoglycemic medications) were asked to consume a solution containing 75 g glucose within a 10-min time window after an overnight fast (≥12 h). Glucose and insulin were measured before the glucose load was administered and 2 h after a participant began consuming the drink.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for Windows, release 11; Chicago, IL). The nonparametric Kruskal-Wallis test was used when comparing continuous variables, and a Fisher's exact test was used for analyzing contingency tables. Logistic regression analysis was used to study the relation of the metabolic syndrome with circulating oxLDL (expressed in mg/dl or as the percent of LDL). The odds ratio (OR) for high oxLDL (>90th percentile of distribution in individuals with no metabolic syndrome components; cutoff value of 1.90 mg/dl or 1.58% of LDL) was determined using individuals without the metabolic syndrome as a reference group. Logistic regression analysis was also used to study the relation of greater waist circumference; higher blood pressure; higher fasting triglycerides, insulin, glucose, and HbA_{1c} levels; and lower HDL cholesterol with the prevalence of high oxLDL. The OR for high oxLDL for participants in the higher quintiles of waist circumference, systolic blood pressure, triglycerides, insulin, glucose, and HbA_{1c} and lower quintiles of HDL cholesterol was determined by comparing these individuals with participants in the lowest quintile of waist circumference, systolic blood pressure, triglycerides, insulin, glucose, and HbA_{1c} and the highest quintile of HDL cholesterol. $P < 0.05$ was considered statistically significant. The relative risk of total CHD and MI according to the metabolic syndrome, compared with individuals without the metabolic syndrome, and according to oxLDL (per quintile) was determined by logistic regression analysis after adjusting for age, sex, ethnicity, and smoking status. An interaction term was fitted to assess whether the metabolic syndrome determined the oxLDL effect on CHD/MI.

RESULTS

Only 4% of the participants in the Health ABC study had no metabolic syndrome components; 58% of subjects had one or two abnormalities and 38% had three or more abnormalities. Prevalence of the metabolic syndrome as defined by ATP III (three or more components) in the Health ABC study was 38 vs. 42% in a similar-aged population in the NHANES III (14). In all, 13% of the Health ABC participants had CHD compared with 20% in the NHANES III cohort. Prevalence of diabetes was 19 vs. 18% and the prevalence of current smoking was 10 vs. 15%, respectively. The mean systolic blood pressure was 136 vs. 142 mmHg; mean waist circumference, 100 vs. 97 cm; mean HDL cholesterol levels, 1.40 vs. 1.33 mmol/l; and mean triglyceride levels, 1.56 and 1.79 mmol/l, for the two study populations, respectively. Mean BMI was 27 kg/m^2 in both the Health ABC cohort and the age-matched NHANES III cohort.

Table 1 summarizes the characteristics of the study cohort, contrasting participants without and with the metabolic syndrome. Compared with those without the metabolic syndrome, participants with the syndrome were more likely to be female and have a heavier BMI (weight in kilograms divided by height in meters) (2), larger waist circumference, higher blood pressure, more glucose intolerance and diabetes, lower HDL cholesterol, and higher triglyceride level. Smoking habits were similar for those with and without the metabolic syndrome. OxLDL levels (absolute levels expressed in mg/dl or relative levels expressed in percent of LDL) were higher in those with the metabolic syndrome (Table 1).

Compared with those without the metabolic syndrome, the OR for high oxLDL (>1.90 mg/dl) in participants with the metabolic syndrome was 1.82 ($P < 0.001$), after adjusting for age, sex, ethnicity, and smoking status (Table 2). No interaction with sex and ethnicity was observed. After

TABLE 1
Characteristics of the study cohort

	Total population	Subjects without metabolic syndrome	Subjects with metabolic syndrome	*P*
n	3,033	1,886	1,147	—
Age (years)	74 ± 2.9	74 ± 2.9	74 ± 2.8	NS
Male (%)	48	52	42	<0.0001
African American (%)	42	42	41	NS
BMI (kg/m^2)	27 ± 4.9	27 ± 4.7	29 ± 4.8	<0.0001
Waist circumference (cm)	100 ± 13	98 ± 13	102 ± 12	<0.0001
High blood pressure (%)	39	35	45	<0.0001
Systolic blood pressure (mmHg)	136 ± 21	134 ± 21	139 ± 21	<0.0001
Diastolic blood pressure (mmHg)	71 ± 12	71 ± 12	72 ± 11	NS
Diabetes (%)	19	7	38	<0.0001
Glucose intolerance (%)*	24	9	49	<0.0001
Fasting glucose (mmol/l)	5.83 ± 1.94	5.33 ± 1.28	6.66 ± 2.55	<0.0001
Fasting insulin (units/ml)	8.55 ± 7.52	7.31 ± 5.99	10.90 ± 9.34	<0.0001
HbA_{1c} (%)	6.37 ± 1.13	6.12 ± 0.83	6.78 ± 1.40	<0.0001
Lipid-lowering drugs (%)	15	13	19	<0.0001
Total cholesterol (mmol/l)	5.25 ± 1.01	5.22 ± 0.096	5.28 ± 1.06	NS
LDL cholesterol (mmol/l)	3.15 ± 0.91	3.15 ± 0.88	3.13 ± 0.93	NS
Low HDL cholesterol (%)	29	10	62	<0.0001
HDL cholesterol (mmol/l)	1.40 ± 0.44	1.53 ± 0.44	1.19 ± 0.36	<0.0001
High triglycerides (%)	30	9.2	66	<0.0001
Triglycerides (mmol/l)	1.56 ± 0.93	1.22 ± 0.48	2.14 ± 1.19	<0.0001
Triglyceride-to-HDL molar ratio	1.31 ± 1.22	0.88 ± 0.50	2.01 ± 1.65	<0.0001
Smoking: former/current (%)	46/10	46/11	46/9.1	NS
Average pack-years among smokers	19 ± 28	19 ± 27	20 ± 21	NS
OxLDL (mg/dl)	1.32 ± 0.74	1.23 ± 0.67	1.45 ± 0.82	<0.0001
OxLDL (% of LDL)	1.09 ± 0.53	1.01 ± 0.46	1.21 ± 0.59	<0.0001

Data are means ± SD. Subjects without and with the metabolic syndrome were compared with the Kruskal-Wallis or χ^2 test. *Glucose intolerance determined according to American Diabetes Association criteria.

further adjusting for LDL cholesterol, the OR was 2.01 ($P < 0.001$) (Table 2). When oxLDL was expressed as the percent of LDL, the adjusted OR for high oxLDL (>1.58%) was 2.56 ($P < 0.001$) (Table 2).

Logistic regression analysis was performed to study the relation between the degree of the different metabolic syndrome components and oxLDL. Compared with those in the lowest quintile and adjusting for age, ethnicity, and LDL cholesterol, the prevalence of high oxLDL was higher in higher quintiles of waist circumference, serum triglycerides, and insulin and glucose levels. Compared with the highest quintile of HDL cholesterol, the prevalence of high oxLDL was higher in lower quintiles of HDL cholesterol. No significant association between blood pressure and oxLDL was observed (Table 3). Compared with the lowest quintile of the triglyceride-to-HDL molar ratio, the OR for high oxLDL was 1.57 (95% confidence interval [CI] 1.01–2.44) in the 2nd quintile and increased to 2.03 (1.33–3.09), 2.45 (1.62–3.71), and 3.74 (2.46–5.67) in the 3rd, 4th, and 5th quintiles, respectively. Compared with the lowest quintile, the OR for high oxLDL in the highest quintile of HbA_{1c} was 1.67 (1.25–2.23), after adjusting for age, sex, ethnicity, lipid levels, smoking status, and glucose and insulin levels.

Compared with participants without the metabolic syndrome and adjusting for age, sex, ethnicity, and smoking status, those with the metabolic syndrome had a 1.69-fold higher risk (95% CI 1.29–2.34) for CHD events. OxLDL did not predict the risk of CHD events (relative risk for participants in highest quintile 1.23; 0.83–1.82). Relative risk ratios for future CHD events for those without CHD at baseline were 1.52 (1.15–2.01) for the metabolic syndrome and 1.45 (0.91–2.34) for oxLDL (highest versus lowest quintile).

Recently, oxLDL was found to be associated with acute coronary events (5,18). We therefore determined the predictive value of oxLDL for new MI events. Table 4 illustrates the relative risk of MI in relation to the metabolic

TABLE 2
Logistic regression analysis of the relation between the metabolic syndrome and prevalence of high circulating oxLDL

	Subjects without metabolic syndrome	Subjects with metabolic syndrome
High oxLDL >1.90 mg/dl	1.00 (—)	1.82 (1.49–2.23)
Adjusted for age, sex, ethnicity, and smoking status		
Adjusted for age, sex, ethnicity, smoking status, and LDL cholesterol	1.00 (—)	2.01 (1.61–2.52)
High oxLDL >1.58% of LDL, adjusted for age, sex, ethnicity, and smoking status	1.00 (—)	2.56 (2.05–3.20)

Data are OR (95% CI). The ORs for high oxLDL (exceeding the 90th percentile of distribution for subjects without the metabolic syndrome) for those with the metabolic syndrome were determined by logistic regression analysis comparing with subjects without the metabolic syndrome.

TABLE 3
ORs of high oxLDL according to the degree of individual metabolic syndrome components

Metabolic syndrome component	Quintile of metabolic syndrome component				
	1st	2nd	3rd	4th	5th
Waist circumference	1 (—)	1.14 (0.80–1.61)	1.22 (0.86–1.72)	0.99 (0.70–1.41)	1.48 (1.05–2.09)
HDL cholesterol	1 (—)	1.42 (0.96–2.10)	1.88 (1.28–2.78)	2.08 (1.42–3.05)	3.10 (2.06–4.65)
Triglycerides	1 (—)	1.47 (0.95–1.88)	1.34 (0.87–2.07)	1.79 (1.17–2.74)	3.12 (2.06–4.73)
Insulin	1 (—)	0.70 (0.46–1.06)	1.12 (0.75–1.66)	1.75 (1.20–2.56)	1.98 (1.36–2.88)
Glucose	1 (—)	1.11 (0.76–1.62)	1.05 (0.71–1.55)	1.29 (0.90–1.86)	2.03 (1.41–2.92)
Blood pressure	1 (—)	1.03 (0.72–1.49)	1.08 (0.76–1.52)	1.04 (0.73–1.47)	1.27 (0.91–1.78)

Data are OR (95% CI). The ORs and 95% CI for high oxLDL for subjects in the highest quintile (lowest quintile for HDL cholesterol) compared with subjects in the lowest quintile (highest quintile for HDL cholesterol) were obtained by logistic regression analysis and adjusted for age, sex, ethnicity, LDL cholesterol, and smoking status.

syndrome and oxLDL as determined by logistic regression analysis and adjusted for age, sex, ethnicity, and smoking status. Those with the metabolic syndrome had a 2.0-fold higher risk. The incidence rate for those with the metabolic syndrome was 5.6 vs. 2.9% ($P < 0.001$) for those without the metabolic syndrome. We also divided the cohort into five groups by levels of oxLDL (Table 4). The incidence of MI was 5.7% in the highest quintile of oxLDL compared with 2.6% ($P < 0.01$) in the lowest quintile. The risk ratio for participants in the highest quintile was 2.25 (95% CI 1.22–4.15). After adjusting for the metabolic syndrome, the risk ratio for participants in the highest quintile of oxLDL was 1.87 (1.00–3.49). There was no significant interaction between the metabolic syndrome and oxLDL, suggesting that the effect of oxLDL on MI was independent of the metabolic syndrome.

DISCUSSION

We have shown for the first time in a population cohort that the metabolic syndrome is associated with a higher fraction of oxLDL and thus with higher levels of circulating oxLDL. This association was consistent across sex and ethnicity. Our data further support the importance of identifying individuals with the metabolic syndrome as a high-risk group for developing CHD. Finally, our study identified the oxidation of LDL as a potential mechanism explaining the increased risk for MI among those with the metabolic syndrome.

Metabolic syndrome components and oxLDL. In elderly individuals, three dominant factors have been identified in the relation between the metabolic syndrome and ischemic heart disease: *1*) the central metabolic factor, comprised of obesity, fasting and nonfasting insulin, and dyslipidemia (HDL cholesterol loading negative and triglycerides loading positive), *2*) the glucose factor, and *3*) the blood pressure factor (19,20). Our study showed a strong association with two out of the three metabolic syndrome factors: the central metabolic syndrome factor, with obesity, dyslipidemia, and insulin resistance, and the glucose factor. The association with these different factors suggests that oxLDL may be generated through several mechanisms.

LDL phenotype and oxLDL. Recently, an association between small LDL particle size and the oxidation of LDL has been shown in healthy middle-aged men (21). In the elderly population, lipids are significantly associated with ischemic heart disease, largely based on the impact of high triglycerides and low HDL cholesterol and less so for LDL cholesterol (20). Although individuals with the metabolic syndrome often have average levels of LDL, they may have qualitative abnormalities. Small, dense LDL particles (22) have been associated with components of the insulin resistance syndrome, including hypertriglyceridemia, low serum HDL cholesterol levels, and diabetes. Also, small, dense LDL particles, which are particularly prone to oxidation, have been proven to be more atherogenic than larger LDL particles (23–26). In the Health ABC study, the prevalence of small, dense LDLs has not been directly studied. It has, however, been shown that 90% of those with a triglyceride-to-HDL molar ratio >1.33 had small, dense LDL (27). In this study, a triglyceride-to-HDL molar ratio >1.33 was a better predictor of elevated levels of oxLDL than high triglycerides and low HDL cholesterol

TABLE 4
Relative risk of MI according to metabolic syndrome or/and high oxLDL

Model 1	
Age	1.01 (0.95–1.07)
Sex	2.55 (1.70–3.81)
Ethnicity	1.03 (0.71–1.49)
Smoking status	1.00 (0.99–1.01)
Metabolic syndrome	2.11 (1.46–3.04)
Model 2	
Age	1.01 (0.95–1.08)
Sex	2.41 (1.61–3.59)
Ethnicity	1.04 (0.72–1.51)
Smoking status	1.00 (0.99–1.01)
OxLDL	
2nd quintile	1.29 (0.66–2.52)
3rd quintile	1.79 (0.96–3.36)
4th quintile	1.82 (0.97–3.42)
5th quintile	2.25 (1.22–4.15)
Model 3	
Age	1.01 (0.95–1.07)
Sex	2.62 (1.78–3.86)
Ethnicity	1.06 (0.73–1.53)
Smoking status	1.00 (0.99–1.01)
Metabolic syndrome	1.97 (1.35–2.86)
OxLDL	
2nd quintile	1.20 (0.61–2.35)
3rd quintile	1.65 (0.88–3.10)
4th quintile	1.63 (0.86–3.07)
5th quintile	1.87 (1.00–3.49)

Data are relative risk ratio (95% CI), determined by logistic regression analysis comparing subjects with to subjects without the metabolic syndrome and/or subjects in higher quintiles to subjects in the lowest quintile of oxLDL.

separately, suggesting an association between the small, dense LDL phenotype and the oxidation of LDL in individuals with the metabolic syndrome.

HDL and oxLDL. Previously, an inverse relation between HDL cholesterol and circulating oxLDL has been shown in healthy middle-aged men (21). This inverse relation can be explained by the antioxidant action of HDL components (28). The HDL-associated enzymes paraoxonase and lecithin:cholesterol acyltransferase prevent LDL oxidation and render LDL resistant to oxidation (29,30). Recently, we used mice with both leptin and LDL receptor deficiency to study the relation of insulin resistance and dyslipidemia with atherosclerosis (31). We demonstrated that the high susceptibility of these mice for atherosclerosis was due to increased oxidative stress, as evidenced by the higher levels of antibodies against oxLDL. Those higher levels resulted from decreased antioxidant defense of HDL related to decreased paraoxonase and lecithin:cholesterol acyltransferase activity. It remains to be investigated whether the decreased antioxidant defense of HDL can explain the inverse relation between HDL cholesterol and oxLDL in individuals with the metabolic syndrome.

Glucose, insulin, HbA_{1c}, and oxLDL. It has been shown in healthy, nondiabetic volunteers that plasma glucose and insulin levels correlate with a higher susceptibility of ex vivo oxidation of LDL (32). Here we have shown that hyperinsulinemia and impaired glycemic control are associated with increased in vivo LDL oxidation, as reflected by the higher prevalence of high oxLDL. It has been demonstrated that impaired glucose tolerance in elderly individuals is associated with the formation of glycoxidation products of LDL that accumulate in human atherosclerotic plaques (33). Not only can glucose induce LDL oxidation, it also can impair the antioxidant properties of serum albumin (34).

Previously, an inverse correlation has been observed between HbA_{1c}, our commonly used clinical index of glycemic control, and the lag time for ex vivo LDL oxidation, a proxy for LDL susceptibility to oxidation (35,36). Here we have shown a relation between HbA_{1c} and in vivo oxLDL using an ELISA that also detects LDL glycoxidation products.

It has been shown that insulin treatment prevents LDL from accelerated in vitro oxidation (37), which suggests a protective effect of insulin against LDL oxidation. Here we demonstrated, however, that hyperinsulinemia is associated with a higher prevalence of having high levels of in vivo oxLDL. Our data are thus in agreement with a synergistic effect of insulin and oxLDL in atherogenesis. Indeed, it has been demonstrated that insulin interacts with cytokines and growth factors in vascular wall cells in concert with oxidized and/or glycated lipoproteins by inducing the expression of monocyte/macrophage specific adhesion molecules such as intracellular adhesion molecule-1 (38).

The metabolic syndrome, oxLDL, and risk of coronary heart disease. As in the Healthy American Women Study and the West of Scotland Coronary Prevention Study (25,39), the modified National Cholesterol Education Program metabolic syndrome definition predicted total CHD and MI risk in the Health ABC cohort. OxLDL did not predict the total CHD risk, but did predict MI. These observations, along with previous research into the association of LDL oxidation with atherosclerosis and CHD (8,10,40,41), provide evidence that LDL oxidation is a common basis for the metabolic syndrome and CHD or, more particularly, for inducing atherothrombotic coronary disease. Previous studies in the hypercholesterolemic miniature pig have suggested that the oxidation of LDL occurs in the arterial wall and not in the circulation (42,43). Recently, Tsimikas et al. (18) demonstrated temporal increases of circulating oxLDL in association with acute coronary syndromes. In aggregate, these data support the hypothesis that an increase in oxLDL reflects plaque instability.

Limitations of this study. This epidemiological study does not give insight into the mechanisms by which the metabolic syndrome and oxLDL might contribute to the development of CHD or plaque instability. Previous studies into the effect of in vitro oxLDL on vascular cell function did not determine the minimal amounts of oxLDL required to alter the redox state of vascular cells. These studies typically use concentrations of oxLDL that are 10-fold higher than those detected in the plasma.

This is the first report of an association between the metabolic syndrome and a high prevalence of high oxLDL in a population-based cohort. This association remained valid after adjusting for LDL cholesterol, sex, and ethnicity. Our data also further support the predictive value of the metabolic syndrome for CHD and suggest that baseline levels of oxLDL add prognostic information concerning future risk for MI.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants N01-AG-6-2103, N01-AG-6-210, and N01-AG-6-2106; the Interuniversitaire Attractiepolen Programma of the Belgian Federal Government (P05/02); and the Fonds voor Wetenschappelijk Onderzoek-Vlaanderen (G.0088.02).

We thank M. Landeloos for excellent technical assistance.

REFERENCES

1. Goldstein JL, Brown MS: Molecular medicine: the cholesterol quartet. *Science* 292:1310–1312, 2001
2. Mertens A, Holvoet P: Oxidized LDL and HDL: antagonists in atherothrombosis. *FASEB J* 15:2073–2084, 2001
3. Ross R: Cell biology of atherosclerosis. *Annu Rev Physiol* 57:791–804, 1995
4. Ehara S, Ueda M, Naruko T, Haze K, Itoh A, Otsuka M, Komatsu R, Matsuo T, Itabe H, Takano T, Tsukamoto Y, Yoshiyama M, Takeuchi K, Yoshikawa J, Becker AE: Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. *Circulation* 103:1955–1960, 2001
5. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D: Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation* 98:1487–1494, 1998
6. Toshima S, Hasegawa A, Kurabayashi M, Itabe H, Takano T, Sugano J, Shimamura K, Kimura J, Michishita I, Suzuki T, Nagai R: Circulating oxidized low density lipoprotein levels: a biochemical risk marker for coronary heart disease. *Arterioscler Thromb Vasc Biol* 20:2243–2247, 2000
7. Holvoet P, Van Cleemput J, Collen D, Vanhaecke J: Oxidized low density lipoprotein is a prognostic marker of transplant-associated coronary artery disease. *Arterioscler Thromb Vasc Biol* 20:698–702, 2000
8. Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, Collen D, Muls E, Van de Werf F: Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 21:844–848, 2001
9. Kopprasch S, Pietzsch J, Kuhlisch E, Fuecker K, Temelkova-Kurktschiev T,

Hanefeld M, Kuhne H, Julius U, Graessler J: In vivo evidence for increased oxidation of circulating LDL in impaired glucose tolerance. *Diabetes* 51:3102–3106, 2002
10. Holvoet P, Harris TB, Tracy RP, Verhamme P, Newman AB, Rubin SM, Simonsick EM, Colbert LH, Kritchevsky SB: Association of high coronary heart disease risk status with circulating oxidized LDL in the well-functioning elderly: findings from the Health, Aging and Body Composition Study. *Arterioscler Thromb Vasc Biol* 23:1444–1448, 2003
11. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L: Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 24:683–689, 2001
12. Trevisan M, Liu J, Bahsas FB, Menotti A: Syndrome X and mortality: a population-based study: Risk Factor and Life Expectancy Research Group. *Am J Epidemiol* 148:958–966, 1998
13. National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Bethesda, MD, National Institutes of Health, 2002 (NIH publ. no. 01-3670)
14. Ford ES, Giles WH, Dietz WH: Prevalence of the metabolic syndrome among US adults: findings from the Third National Health and Nutrition Examination Survey. *JAMA* 287:356–359, 2002
15. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density-lipoprotein cholesterol in plasma without use of the ultracentrifuge. *Clin Chem* 18:449–502, 1972
16. Holvoet P, Donck J, Landeloos M, Brouwers E, Luijtens K, Arnout J, Lesaffre E, Vanrenterghem Y, Collen D: Correlation between oxidized low density lipoproteins and von Willebrand factor in chronic renal failure. *Thromb Haemost* 76:663–669, 1996
17. Holvoet P, Stassen JM, Van Cleemput J, Collen D, Vanhaecke J: Oxidized low density lipoproteins in patients with transplant-associated coronary artery disease. *Arterioscler Thromb Vasc Biol* 18:100–107, 1998
18. Tsimikas S, Bergmark C, Beyer RW, Patel R, Pattison J, Miller E, Juliano J, Witztum JL: Temporal increases in plasma markers of oxidized low-density lipoprotein strongly reflect the presence of acute coronary syndromes. *J Am Coll Cardiol* 41:360–370, 2003
19. Lindblad U, Langer RD, Wingard DL, Thomas RG, Barrett-Connor EL: Metabolic syndrome and ischemic heart disease in elderly men and women. *Am J Epidemiol* 153:481–489, 2001
20. Shepherd J: Issues surrounding age: vascular disease in the elderly. *Curr Opin Lipidol* 12:601–609, 2001
21. Sigurdardottir V, Fagerberg B, Hulthe J: Circulating oxidized low-density lipoprotein (LDL) is associated with risk factors of the metabolic syndrome and LDL size in clinically healthy 58-year-old men (AIR study). *J Intern Med* 252:440–447, 2002
22. Krauss RM, Burke DJ: Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J Lipid Res* 23:97–104, 1982
23. Chancharme L, Therond P, Nigon F, Lepage S, Couturier M, Chapman MJ: Cholesteryl ester hydroperoxide lability is a key feature of the oxidative susceptibility of small, dense LDL. *Arterioscler Thromb Vasc Biol* 19:810–820, 1999
24. Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Despres JP: Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men: prospective results from the Quebec Cardiovascular Study. *Circulation* 95:69–75, 1997
25. Ridker PM, Buring JE, Cook NR, Rifai N: C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14,719 initially healthy American women. *Circulation* 107:391–397, 2003
26. Tracy RP, Lemaitre RN, Psaty BM, Ives DG, Evans RW, Cushman M, Meilahn EN, Kuller LH: Relationship of C-reactive protein to risk of cardiovascular disease in the elderly: results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol* 17:1121–1127, 1997
27. Boizel R, Benhamou PY, Lardy B, Laporte F, Foulon T, Halimi S: Ratio of triglycerides to HDL cholesterol is an indicator of LDL particle size in patients with type 2 diabetes and normal HDL cholesterol levels. *Diabetes Care* 23:1679–1685, 2000
28. Navab M, Berliner JA, Subbanagounder G, Hama S, Lusis AJ, Castellani LW, Reddy S, Shih D, Shi W, Watson AD, Van Lenten BJ, Vora D, Fogelman AM: HDL and the inflammatory response induced by LDL-derived oxidized phospholipids. *Arterioscler Thromb Vasc Biol* 21:481–488, 2001
29. Bielicki JK, Forte TM: Evidence that lipid hydroperoxides inhibit plasma lecithin:cholesterol acyltransferase activity. *J Lipid Res* 40:948–954, 1999
30. Watson AD, Berliner JA, Hama SY, La Du BN, Faull KF, Fogelman AM, Navab M: Protective effect of high density lipoprotein associated paraoxonase: inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest* 96:2882–2891, 1995
31. Mertens A, Verhamme P, Bielicki JK, Phillips MC, Quarck R, Verreth W, Stengel D, Ninio E, Navab M, Mackness B, Mackness M, Holvoet P: Increased low-density lipoprotein oxidation and impaired high-density lipoprotein antioxidant defense are associated with increased macrophage homing and atherosclerosis in dyslipidemic obese mice: LCAT gene transfer decreases atherosclerosis. *Circulation* 107:1640–1646, 2003
32. 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* 152:203–208, 2000
33. Imanaga Y, Sakata N, Takebayashi S, Matsunaga A, Sasaki J, Arakawa K, Nagai R, Horiuchi S, Itabe H, Takano T: In vivo and in vitro evidence for the glycoxidation of low density lipoprotein in human atherosclerotic plaques. *Atherosclerosis* 150:343–355, 2000
34. Bourdon E, Loreau N, Blache D: Glucose and free radicals impair the antioxidant properties of serum albumin. *FASEB J* 13:233–244, 1999
35. Liguori A, Abete P, Hayden JM, Cacciatore F, Rengo F, Ambrosio G, Bonaduce D, Condorelli M, Reaven PD, Napoli C: Effect of glycaemic control and age on low-density lipoprotein susceptibility to oxidation in diabetes mellitus type 1. *Eur Heart J* 22:2075–2084, 2001
36. Makimattila S, Liu ML, Vakkilainen J, Schlenzka A, Lahdenpera S, Syvanne M, Mantysaari M, Summanen P, Bergholm R, Taskinen MR, Yki-Jarvinen H: Impaired endothelium-dependent vasodilation in type 2 diabetes: relation to LDL size, oxidized LDL, and antioxidants. *Diabetes Care* 22:973–981, 1999
37. Kondo A, Manabe M, Saito K, Maekawa M, Kanno T: Insulin treatment prevents LDL from accelerated oxidation in patients with diabetes. *J Atheroscler Thromb* 9:280–287, 2002
38. Sampson MJ, Davies IR, Brown JC, Ivory K, Hughes DA: Monocyte and neutrophil adhesion molecule expression during acute hyperglycemia and after antioxidant treatment in type 2 diabetes and control patients. *Arterioscler Thromb Vasc Biol* 22:1187–1193, 2002
39. Sattar N, Gaw A, Scherbakova O, Ford I, O'Reilly DS, Haffner SM, Isles C, Macfarlane PW, Packard CJ, Cobbe SM, Shepherd J: Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary Prevention Study. *Circulation* 108:414–419, 2003
40. Hulthe J, Fagerberg B: Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR Study). *Arterioscler Thromb Vasc Biol* 22:1162–1167, 2002
41. Hulthe J, Fagerberg B: Circulating oxidized LDL is associated with increased levels of cell-adhesion molecules in clinically healthy 58-year old men (AIR study). *Med Sci Monit* 8:CR148–CR152, 2002
42. Holvoet P, Theilmeier G, Shivalkar B, Flameng W, Collen D: LDL hypercholesterolemia is associated with accumulation of oxidized LDL, atherosclerotic plaque growth, and compensatory vessel enlargement in coronary arteries of miniature pigs. *Arterioscler Thromb Vasc Biol* 18:415–422, 1998
43. Verhamme P, Quarck R, Hao H, Knaapen M, Dymarkowski S, Bernar H, Van Cleemput J, Janssens S, Vermylen J, Gabbiani G, Kockx M, Holvoet P: Dietary cholesterol withdrawal reduces vascular inflammation and induces coronary plaque stabilization in miniature pigs. *Cardiovasc Res* 56:135–144, 2002