Oxidized low-density lipoprotein in post-menopausal women

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Abstract

Oxidized low density lipoprotein (oxLDL) leads to atherosclerosis and cardiovascular disease, the most frequent cause of death worldwide. After menopause, lipid and lipoprotein metabolism changes and women are at greater risk of cardiovascular disease compared to fertile women. Aim of this study was to determine the prevalence of serum oxLDL in postmenopausal women and to identify possible associations of clinical and laboratory features with oxLDL in these patients.

After clinical examination and completing a clinical questionnaire, an ultrasound examination of both carotid arteries was conducted and blood was drawn from 533 postmenopausal women. oxLDL concentration was determined using $^1$H-NMR spectroscopy.

Oxidized LDL was detected in 12.4% (95%CI 9.7 – 15.5) of post-menopausal women with a median of 0.18 mg dL$^{-1}$ (IQR 0.10 – 0.43). Although intima-media-thickness did not differ, post-menopausal women with serous oxLDL had more often atherosclerotic plaques compared to women without oxLDL (6/66 vs. 0/467; p < 0.01). Higher concentrations of high density lipoprotein, impaired glucose intolerance, and diastolic blood pressure were independently associated with the occurrence of oxLDL. If oxLDL was present, higher HDL and glucose intolerance were associated with higher concentrations of oxLDL. In contrast, higher blood urea concentrations were associated with lower concentrations of oxLDL.

This study presents the prevalence and concentration of oxLDL in post-menopausal women and demonstrates that oxLDL concentration can be quantified by $^1$H-NMR spectroscopy in large patient samples. The data suggest that oxLDL may
be a biomarker for incipient atherosclerotic changes in post-menopausal women. In contrary to the association of dyslipoproteinemia and diabetes, higher blood urea concentrations were associated with lower concentrations of oxLDL.
Introduction

The World Health Organization reports that cardiovascular disease (CVD) is the most frequent cause of death worldwide.\footnote{1} Despite tremendous efforts in implementing prevention programs, finding new treatment options, and investing billions of dollars in research, cardiovascular deaths are still representing 30\% of all global deaths. The WHO projects that about 25 million people will die from CVD in 2030.\footnote{1}

Oxidized low density lipoprotein (oxLDL) plays a major role in the pathogenesis of atherosclerosis which leads to CVD. All major cardiovascular risk factors cause oxidative stress: diabetes,\footnote{2} hypertension,\footnote{3} dyslipoproteinemia,\footnote{4} and smoking,\footnote{5} while oxidative stress induces the oxidation of low density lipoprotein. OxLDL transverses the endothelium via the LOX-1 receptor and is phagocytized by macrophages. Subsequently, these macrophages transform into foam cells and accumulate to fatty streaks. Fatty streaks are converted via the formation of a fibrous capsule into atheromas and atherosclerotic plaques.\footnote{6}

Women after menopause are at greater risk for CVD than fertile women.\footnote{7} Lipid and lipoprotein metabolism changes and postmenopausal women are exposed to more oxidative stress than fertile women.\footnote{8} In fact, few studies with small sample sizes have shown that postmenopausal women have elevated serum concentrations of oxLDL.\footnote{8,9}

Therefore, the primary aim of this study was to determine the prevalence of serum oxLDL in a large sample of postmenopausal women. Secondary aim of the
study was to identify possible associations of clinical and laboratory features with serum oxLDL in postmenopausal women.
Methods

Patients

The ethics board of the Ruhr University of Bochum (Germany) approved the study (registration number 1343). Patients were recruited through a random sample of 6,289 postmenopausal women of the population-based cohort from the Bochumer Postmenopausal Study.\textsuperscript{10} Women were considered postmenopausal if they indicated to have stopped menstruating for at least one year. Patients were excluded, if menopause occurred \( \geq 10 \) years before questioning or were under hormone replacement therapy or corticoid medication. 549 patients were consecutively enrolled in this substudy agreeing to give a blood sample after written informed consent. Of the 549 included patients, 16 were not included for missing data, leaving the data of 533 patients to be analyzed.

Clinical questionnaire and anthropometric measures

Patients filled out a questionnaire regarding atherosclerosis risk factors including age, weight, height, history of myocardial infarction, stroke, diabetes, hypertension, hypercholesterolemia, peripheral arterial disease, kidney dysfunction, medication, smoking and eating habits.

After completion of the questionnaire, patients’ body weight was measured to the nearest kilogram and patients’ height to the nearest centimeter. Body mass index (BMI) was calculated as weight/height\(^2\) (kg/m\(^2\)). Blood pressure was measured with a mercury sphingomanometer after 5 minutes in the sitting position. Patients were then drawn blood for biochemical analysis, an oral glucose tolerance test was administered, and an ultrasound of the carotid arteries was conducted.
Biochemical analyses

The blood sample was taken between 7 a.m. and 9 a.m. after 12 hours of fasting. The blood sample was used to determine a full blood cell count and the concentrations of fasting glucose, homocystein, total cholesterol, low density lipoprotein, high density lipoprotein, triglycerides, urea, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, electrolytes, total protein and albumin. Additionally, an aliquot of a whole-blood sample was immediately centrifuged for 20 minutes at 5,000 rpm to obtain serum for $^1$H-NMR spectroscopic analysis prepared immediately for $^1$H-NMR spectroscopy as described below. Patients subsequently performed an oral glucose tolerance test according to WHO standards and another blood glucose was measured 120 minutes after ingestion of the 75g carbohydrates solution (Dextro OGT, Hoffmann-LaRoche, Germany). Glomuerular Filtration rate was estimated using the “Modification of Diet in Renal Disease” (MDRD) formula.

Scanning for intima-media-thickness and artheroslerotic plaques

The intima-media-thickness (IMT) was scanned using a 7.5 MHz linear-array imaging probe on a Toshiba CoreVision Pro SSA 350A (Japan). IMT was measured on both common carotid arteries at 1.0 cm, 1.5 cm and 2.0 cm and proximal of the carotid bifurcation and averaged as mean for each side. The maximum of both sides ($\text{IMT}_{\text{max}}$) was used in the multivariate model. In addition, the physician scanned the right and left common carotid arteries, the carotid bulbs, and the internal carotid arteries for atherosclerotic plaques.
**1H-NMR spectroscopic measurement of oxLDL**

1H-NMR spectroscopy was used for measuring oxLDL concentrations because it allows a fast sample processing and sensitive and accurate measurements. 1H-NMR spectroscopy was conducted as previously described\(^1\) using a serial dilution to obtain calibration measurements. We separated lipids from serum\(^2\) and consecutively isolated chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and low-density protein (LDL) through a discontinuous potassium bromide gradient as described by Havel and colleagues\(^3\). Protein concentration was determined according to the method described by Lowry and colleagues\(^4\). After sodium perchlorate dialysis the isolated and purified LDL was incubated with copper sulfate (CuSO\(_4\)) for 24 hours at 4°C resulting in oxLDL at a defined concentration. A ten-level serial dilution was fabricated and 1H-NMR spectra were obtained. The three characteristic resonances of oxLDL correlated significantly with the concentration of oxLDL, the relative resonance intensity at 1.17 ppm correlated best with the concentration of oxLDL \((r^2 = 0.983; p < 0.01)\). Thus, the regression line’s slope represents the concentration of oxLDL per arbitrary unit (AU) in relation to standard TSP.

**Statistics**

Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov-test. Normally distributed variables are presented as mean ± standard deviation, not normally distributed values as median and quartiles; categorical variables are presented in percent and 95% confidence interval. The probability of a type I error below 0.05 was considered to be statistically significant.

Univariate testing was done using the Mann-Whitney-U-Test and Fisher’s Exact Test. For multivariate testing we calculated two models. The first model was a
binary logistic model to assess associations of covariates with the occurrence of oxLDL in patients' serum, the second model was a linear model to assess the covariates influence on the concentration of oxLDL. Covariates were selected for inclusion in each of the two final models if they were selected in either a forward stepwise inclusion process with a cut-off at $p < 0.05$ or a backward elimination model with a cut-off at $p < 0.10$. 
Results

Using $^1$H-NMR spectroscopy, we detected oxLDL in 66 of 533 (12.4%, 95%CI 9.7 – 15.5) sera of postmenopausal women with a median of 0.18 mg dL$^{-1}$ (IQR 0.10 – 0.43) and a range of 0.02 to 18.10 mg dL$^{-1}$. Basic demographic and laboratory data of the examined collective are shown in Table 1. Figure 1 shows a spectrum of LDL (Figure 1.A) and a spectrum of oxLDL (Figure 1.B) with its characteristic resonances at 1.17ppm, 1.18ppm and 1.20ppm.

Maximum intima-media-thickness was 0.67 mm (IQR 0.60 – 0.73). Although maximal intima-media-thickness did not differ between patients with oxLDL and patients without oxLDL (0.67 [IQR 0.62 – 0.73] vs. 0.67 [IQR 0.60 – 0.70], p = 0.28; Figure 2.A), atherosclerotic plaques were significantly more often detected in the common carotid artery in those patients whose serum contained oxLDL (6/66 vs. 0/467, p < 0.01; Figure 2.B), resulting a relative risk to have atherosclerotic plaques of 8.8 (95%CI 6.9 – 11.1).

Of all the variables tested, there were three variables correlating with the occurrence of oxLDL: none of the questionnaire, none of the anthropometric measures, and three clinical chemistry parameters: concentration of HDL ($r_{\text{HDL}} = -0.13$, $p < 0.01$), LDL/HDL ($r_{\text{LDL/HDL}} = -0.10$, $p = 0.03$), and blood urea ($r_{\text{urea}} = 0.10$, $p = 0.03$).

These results were confirmed in the multivariate model. The occurrence of oxLDL was independently associated with the concentration of HDL, the glucose concentration at both time points of the OGTT (0 and 120 min) and diastolic blood pressure (Table 2.A): An increase of 10 mg dL$^{-1}$ in HDL concentration leads to a an
1.2-fold risk for the presence of oxLDL, a 10 mg dL\(^{-1}\) higher blood glucose before the OGTT to an 0.7-fold, a 10 mg dL\(^{-1}\) higher blood glucose after the OGTT to a 1.1-fold, and a higher diastolic blood pressure of 10 mmHg to a 1.03-fold risk for the occurrence for detecting oxLDL in the serum.

Of all the variables tested, there were four variables correlating with the concentration of oxLDL: from the questionnaire the question whether the patient had been diagnosed diabetic, none from the anthropometric measures, and the clinical chemistry parameters blood urea concentration \((r_{\text{urea}} = 0.36, p < 0.01)\), HDL concentration \((r_{\text{urea}} = 0.49, p < 0.01)\), and estimated GFR \((r_{\text{GFR}} = 0.33, p < 0.01)\).

In the multivariate analysis, these results were substantiated since all three variables were included in the final model. In addition, blood glucose at 120 min in the OGTT an BMI were included in this model but did not reach statistical significance. The final model is summarized in Table 2.B. Ultimately, an 10 mg dL\(^{-1}\) more of HDL was associated with a 3.3 mg dL\(^{-1}\) higher concentration of oxLDL \((\text{beta}_{\text{HDL}} = 0.326)\), 10 mg dL\(^{-1}\) more blood glucose concentration was associated with a higher oxLDL concentration of 1.9 mg dL\(^{-1}\), and a 2 mg dL\(^{-1}\) higher blood urea concentration was associated with a 0.5 mg dL\(^{-1}\) lower concentration of oxLDL.
Discussion

The prevalence of oxLDL in postmenopausal women ≥ 10 years after meno-
pause without hormone replacement therapy is 12% and its concentration in median
0.18 mg dL\(^{-1}\). Although women with serum oxLDL did not differ in intima-media-
thickness from those showing no oxLDL, we detected atherosclerotic plaques more
often in these women. We found that dyslipoproteinemia, impaired glucose tolerance,
and diastolic blood pressure were associated with the occurrence of oxLDL in post-
menopausal women. Higher oxLDL concentrations were associated with higher HDL
concentrations and a higher blood glucose 120 minutes after OGTT, lower oxLDL
concentration with higher blood urea concentrations were associated with more fre-
quent detection of oxLDL and with higher concentration.

In \(^1\)H-NMR spectroscopy analysis, the quantification of oxLDL by assessing
the amplitudes of resonances is based on the number of oxidatively modified moie-
ties in the LDL particles, rather than based on the concentration of oxLDL particles.\(^{11}\)
Antibody-based tests – as common alternative – may (1) loose reactivity with anti-
gens that have been minimally perturbed, (2) may show unexpected cross-reactions
with unrelated antigens, and (3) often only work with fractionated samples and are
therefore time-consuming. \(^1\)H-NMR spectroscopy on the other hand offers high
specificity to distinguish molecules based on their chemical structure, and measure-
ments can be carried out quickly without pretreatment of the sera. While \(^1\)H-NMR
spectroscopy is supposedly more accurate, the comparability of oxLDL concentration
measurements in mg L\(^{-1}\) is limited compared to chromatography- and antibody-based
assays measuring activity in U L\(^{-1}\) compared to mg dL\(^{-1}\).\(^{8,9,15,16}\)
The atherosclerotic risk of post-menopausal women is 3.4-times higher compared with pre-menopausal women and intima-media-thickness has been shown to be a valid predictor of cardiovascular events in these patients. Surprisingly, the median intima-media-thickness of our sample was within the normal range for women of equal age. In women with detected oxLDL, both normal intima-media-thicknesses and an increased prevalence of atherosclerotic plaques were found compared with women without oxLDL. Although our results support the findings of Wegner and colleagues who showed oxLDL accelerates atherosclerotic plaque formation, our data challenge the predictive value of intima-media-thickness in special clinical settings: oxLDL may be a better marker for incipient carotid atherosclerosis in post-menopausal women than intima-media-thickness and should be further investigated.

Surprisingly, women with detected oxLDL had a higher concentration of serum HDL. Earlier studies showed that higher oxLDL levels can be associated with higher HDL concentrations under certain circumstances. In animal studies, using LDL receptor null mice Hedrick et al. showed that feeding of an atherogenic diet caused a dramatic decrease in plasma paraoxonase activity and mass without a change in hepatic mRNA levels. The decreased paraoxonase activity was related to an increase in plasma and HDL lipid hydroperoxides. Since paraoxonase hydrolyses lipid peroxides as oxLDL, an increased oxLDL may be observed in some patients.

In this cohort of postmenopausal women, an impaired glucose tolerance was associated with higher oxLDL serum concentrations. Oxidative stress has long been associated with type 2 diabetes mellitus: Oxidative stress has been associated with diabetes, and the concentration of antibodies against oxLDL is independently associated with diabetes. Vice versa, oxLDL serum levels increase with duration of diabetes despite well controlled LDL levels. Our results support the known associa-
tion between oxidative stress, diabetes and elevated oxLDL levels. A limitation of this study is the absence of data regarding actual oxidative stress. This could have led to further insights regarding the formation oxLDL in the population of post-menopausal women.

In addition, a higher blood urea concentration was associated with a lower concentration of oxLDL in this study. The estimated GFR was only univariately correlating with the oxLDL concentration in our data. Whereas oxidative stress in chronic kidney disease is well investigated –especially in end-stage renal disease patients undergoing hemodialysis– little is known about the interaction between kidney function and oxidative stress in patients with minor kidney impairment. In end-stage renal disease patients, hemodialysis reduces oxLDL concentration despite hemococoncentration and the highly pro-oxidant milieu of hemodialysis, yet higher oxLDL concentrations remain associated with an increased risk for cardiovascular events and related death in these patients. We have recently shown that uremic toxins inhibit a pro-oxidative enzyme: NADPH oxidase (unpublished data). The association of higher blood urea concentration –as surrogate parameter for uremia– and lower oxLDL in post-menopausal women concurs with these findings suggesting the possibility that renal impairment could in fact be protective against oxidative stress.

**Perspectives**

In conclusion, we have shown that $^1$H-NMR spectroscopy is a feasible method to determine the concentration of oxLDL in a large number of human sera. Post-menopausal patients with circulating oxLDL had more often atherosclerotic plaques yet the same intima-media-thickness compared with patients without oxLDL. Measur-
ing oxLDL may therefore be a more appropriate marker than intima-media-thickness for assessment and prediction of atherosclerotic lesions in postmenopausal women.

**Acknowledgements**

The authors were supported by grants from the Federal Ministry of Education and Research (NGFN/01GR0807; 13920B), a grant by the Else-Kroener-Foundation (VJ), Sonnenfeld-Foundation and by grant “HEALTH 2011.2.4.2-2” to “Mascara” (278249) from the European Union.

**Sources of Funding**

The study was solely funded by inner-departmental funds.

**Conflict of Interest**

See questionnaire filled out online
References


<table>
<thead>
<tr>
<th></th>
<th>No oxLDL (n = 467)</th>
<th>oxLDL (n = 66)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>54.7 [51.0 – 59.5]</td>
<td>56.4 [50.2 – 60.7]</td>
<td>0.36</td>
</tr>
<tr>
<td>BMI, kg m$^{-2}$</td>
<td>26.1 [23.3 – 29.8]</td>
<td>26.0 [21.7 – 30.5]</td>
<td>0.24</td>
</tr>
<tr>
<td>History of Diabetes</td>
<td>3.9% (n = 18)</td>
<td>9.1% (n = 6)</td>
<td>0.10</td>
</tr>
<tr>
<td>History of MI</td>
<td>0.2% (n = 1)</td>
<td>0.0% (n = 0)</td>
<td>0.71</td>
</tr>
<tr>
<td>History of Stroke</td>
<td>1.3% (n= 6)</td>
<td>0.0% (n = 0)</td>
<td>0.35</td>
</tr>
<tr>
<td>Smoking</td>
<td>30.8% (n = 144)</td>
<td>31.8% (n = 21)</td>
<td>0.89</td>
</tr>
<tr>
<td>Creatinine, mg dl$^{-1}$</td>
<td>0.8 [0.7 – 0.8]</td>
<td>0.8 [0.6 – 0.9]</td>
<td>0.98</td>
</tr>
<tr>
<td>Urea, mg ml$^{-1}$</td>
<td>14 [12 – 17]</td>
<td>14 [11 – 17]</td>
<td>*&lt; 0.05</td>
</tr>
<tr>
<td>Homocystein, μmol l$^{-1}$</td>
<td>10 [8 – 11]</td>
<td>9 [8 – 12]</td>
<td>0.69</td>
</tr>
<tr>
<td>Triglyceride, mg dl$^{-1}$</td>
<td>110 [75 – 149]</td>
<td>90 [67 – 153]</td>
<td>0.17</td>
</tr>
<tr>
<td>Cholesterol, mg dl$^{-1}$</td>
<td>235 [215 – 265]</td>
<td>232 [212 – 261]</td>
<td>0.58</td>
</tr>
<tr>
<td>LDL, mg dl$^{-1}$</td>
<td>152.8 ± 36.4</td>
<td>146.7 ± 37.5</td>
<td>0.23</td>
</tr>
<tr>
<td>HDL, mg dl$^{-1}$</td>
<td>63.0 ± 15.9</td>
<td>69.8 ± 21.3</td>
<td>*0.02</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>2.44 [1.89 – 3.10]</td>
<td>2.26 [1.65 – 2.71]</td>
<td>*0.02</td>
</tr>
<tr>
<td>Glucose OGTT$_{100}$, mg dl$^{-1}$</td>
<td>96 [90 – 104]</td>
<td>95 [87 – 100]</td>
<td>0.13</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value 1 (95% CI)</td>
<td>Value 2 (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>-----------------------------------</td>
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</tr>
<tr>
<td>Glucose OGTT&lt;sub&gt;t=120&lt;/sub&gt;, mg dl&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>90 [77 - 110]</td>
<td>93 [78 - 116]</td>
<td>0.39</td>
</tr>
<tr>
<td>Delta OGTT, mg dl&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>-4 [-18 – 11]</td>
<td>1 [-18 – 23]</td>
<td>0.16</td>
</tr>
<tr>
<td>IFG&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>8.1% (n = 38)</td>
<td>6.0% (n = 4)</td>
<td>0.89</td>
</tr>
<tr>
<td>IGT&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>5.1% (n = 24)</td>
<td>4.5% (n = 3)</td>
<td>0.43</td>
</tr>
<tr>
<td>Diabetes according to OGTT</td>
<td>3.6% (n = 17)</td>
<td>4.5% (n = 3)</td>
<td>0.73</td>
</tr>
<tr>
<td>HbA&lt;sub&gt;1c&lt;/sub&gt;, %</td>
<td>5.3 [5.1 – 5.6]</td>
<td>5.2 [5.0 – 5.7]</td>
<td>0.37</td>
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<tr>
<td>Drug history</td>
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<tr>
<td>Antihypertensives</td>
<td>29.1% (n = 136)</td>
<td>24.2% (n = 16)</td>
<td>0.46</td>
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<tr>
<td>Lipid-lowering agents</td>
<td>8.6% (n = 40)</td>
<td>7.6% (n = 5)</td>
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<tr>
<td>Acetylsalicylic Acid</td>
<td>10.9% (n = 51)</td>
<td>9.1% (n = 6)</td>
<td>0.83</td>
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<tr>
<td>IMT&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;c)&lt;/sup&gt;, mm</td>
<td>0.67 [0.60 – 0.70]</td>
<td>0.67 [0.62 – 0.73]</td>
<td>0.28</td>
</tr>
<tr>
<td>Atherosclerotic plaques&lt;sup&gt;d)&lt;/sup&gt;</td>
<td>0% (n = 0)</td>
<td>9.1% (n = 6)</td>
<td>*&lt;0.01</td>
</tr>
</tbody>
</table>

<sup>a)</sup>impaired fasting glucose; <sup>b)</sup>impaired glucose tolerance; <sup>c)</sup>maximal intima media thickness; <sup>d)</sup>by ultrasonic scan; *statistically significant, Fischer’s exact test or Mann-Whitney-U test.
Table 2: Summary of regression models: (A) binary logistic regression for the occurrence of oxLDL in serum of 533 postmenopausal women, (B) linear regression for the concentration of oxLDL the 66 postmenopausal women with serum oxLDL present ($r^2 = 0.47$). For both models, all variables listed in Table 1 were considered as covariates; covariates were selected for inclusion in the final model if they were either selected in a forward stepwise inclusion process with a cut-off at $p < 0.05$ or a backward elimination process with a cut-off at $p < 0.10$.

### A

<table>
<thead>
<tr>
<th></th>
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<th>SEₜ</th>
<th>OR (95%CI)ᵃ</th>
<th>p</th>
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<tr>
<td>Constant</td>
<td>-1.72</td>
<td>1.51</td>
<td>n/a</td>
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<td>HDLᵇ, mg dl⁻¹</td>
<td>0.020</td>
<td>0.009</td>
<td>1.020 (1.002 – 1.039)</td>
<td>*0.04</td>
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<tr>
<td>OGTTᶜbaseline, mg dl⁻¹</td>
<td>-0.032</td>
<td>0.015</td>
<td>0.968 (0.940 – 0.998)</td>
<td>*0.04</td>
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<tr>
<td>OGTTᶜ120min, mg dl⁻¹</td>
<td>0.012</td>
<td>0.005</td>
<td>1.012 (1.002 – 1.022)</td>
<td>*0.02</td>
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<tr>
<td>Diastolic BPᵈ, mmHg</td>
<td>0.003</td>
<td>0.002</td>
<td>1.003 (1.000 – 1.007)</td>
<td>*0.04</td>
</tr>
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</table>

ᵃOdds ratio with 95% confidence interval; ᵇhigh density lipoprotein; ᵇoral glucose tolerance test; ᵇblood pressure; *statistically significant.

### B

<table>
<thead>
<tr>
<th></th>
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<td>4.002</td>
<td>-1.761</td>
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<td>BMI, kg m⁻²</td>
<td>0.001</td>
<td>0.039</td>
<td>0.005</td>
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<tr>
<td>HDLᵃ, mg dl⁻¹</td>
<td>0.130</td>
<td>0.049</td>
<td>1.328</td>
<td>2.635</td>
<td>*0.01</td>
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<tr>
<td>LDLᵇ, mg dl⁻¹</td>
<td>0.051</td>
<td>0.025</td>
<td>1.039</td>
<td>1.998</td>
<td>*0.05</td>
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<tr>
<td>HDL * LDL, mg² dl⁻²</td>
<td>-0.001</td>
<td>0.000</td>
<td>-1.334</td>
<td>-2.059</td>
<td>*0.04</td>
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<tr>
<td>OGTTᶜ120min, mg dl⁻¹</td>
<td>-0.017</td>
<td>0.006</td>
<td>0.372</td>
<td>2.963</td>
<td>*&lt;0.01</td>
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<td>Urea</td>
<td>-0.254</td>
<td>0.069</td>
<td>-0.477</td>
<td>-3.691</td>
<td>*&lt;0.01</td>
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ᵃHigh density lipoprotein; ᵇlow density lipoprotein; ᵇoral glucose tolerance test; *statistically significant.
**Figure Legends**

**Figure 1:** $^1$H-NMR spectroscopy oxLDL. The $^1$H-NMR spectra of LDL (a) and oxidated LDL (b) significantly vary in three characteristic peaks at 1.17 ppm, 1.18 ppm and 1.20 ppm (marked with an asterisk).

**Figure 2:** Ultrasound-detected atherosclerotic vessel alterations in postmenopausal women by $^1$H-NMR detection of oxidized LDL: (a) maximum Intima-Media-Thickness ($\text{IMT}_{\text{max}}$) in women without ($n = 469$) and with ($n = 66$) oxidized LDL; (b) atherosclerotic plaques in women without ($n = 469$) and with ($n = 66$) oxidized LDL.
Figure 2

(a) Box plot showing maximum intima-media-thickness (mm) between no oxLDL and oxLDL groups. The p-value is 0.28.

(b) Bar graph depicting frequency (%) between no oxLDL and oxLDL groups. The p-value is less than 0.01, indicating a significant difference with 9.1% plaques in the oxLDL group.