Measures of Oxidized Low-Density Lipoprotein and Oxidative Stress Are Not Related and Not Elevated in Otherwise Healthy Men With the Metabolic Syndrome

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**Objective**—The metabolic syndrome predisposes to the development of cardiovascular diseases. Oxidative stress and elevated circulating oxidized low-density lipoprotein (LDL) concentrations are related to cardiovascular disease and proposed to be features of the metabolic syndrome. F2-isoprostanes are lipid peroxidation products and considered the most reliable biomarkers of oxidative stress.

**Methods and Results**—Plasma oxidized LDL (oxLDL) and urinary 8-iso-prostaglandin F2α (8-iso-PGF2α, the major F2-isoprostane) were analyzed in a cross-sectional study of 289 healthy men (62 to 64 years of age). Individuals completed a 7-day dietary record, and fasting plasma insulin, lipid, and lipoprotein concentrations, LDL particle size, and inflammatory markers were determined. National Cholesterol Education Program/Adult Treatment Panel III (NCEP/ATPIII) criteria were used to define the metabolic syndrome and individuals were grouped according to the number of risk factors for the metabolic syndrome (0, [n=88; 30%]; ≧1, [n=179; 62%], metabolic syndrome [n=22; 8%]). Group comparisons revealed no differences for oxLDL, 8-iso-PGF2α, or reported intake of macronutrients, whereas C-reactive protein and interleukin-6 were increased in the metabolic syndrome. LDL cholesterol strongly determined oxLDL in univariate and multivariate analysis, but no relationship to 8-iso-PGF2α was found. In turn, 8-iso-PGF2α was related to reported intake of fat, fatty acids, and dietary antioxidants.

**Conclusions**—There were no increases in plasma oxLDL or measures of oxidative stress (urinary 8-iso-PGF2α) in these otherwise healthy 63-year-old men with the metabolic syndrome. Furthermore, no relationship between oxLDL and 8-iso-PGF2α was found, but our results suggest a role for dietary factors in oxidative stress. (*Arterioscler Thromb Vasc Biol. 2005;25:2580-2586.*)

**Key Words:** metabolic syndrome ■ oxLDL ■ isoprostanes ■ oxidative stress ■ diet

Today, the metabolic syndrome is one of the major public health concerns as its prevalence increases worldwide with a subsequent predisposition to type 2 diabetes and cardiovascular disease. The atherogenic lipoprotein phenotype is a well-established link between the metabolic syndrome and atherosclerotic disease, but also, increased oxidative stress and a proinflammatory state have been suggested as common features of these conditions.1,2 Reactive oxygen species (ROS) can induce tissue damage and lipoprotein modification, thereby promoting the development of various diseases. Increased oxidative stress has been suggested as an early event in the development of the metabolic syndrome and, as such, might contribute to disease progression.3

Another aspect of oxidative modification is the presence of oxidized low-density lipoprotein (LDL). Oxidative modification of LDL can generate “fully oxidized LDL” (recognized by scavenger receptors) and “minimally modified LDL” (not recognized by scavenger receptors). Both of these have been detected in atherosclerotic plaques, but only the latter exists in substantial amounts in the circulation. The concentration of circulating oxidized LDL (oxLDL) has shown relationships with atherosclerotic disease, and some studies indicate oxLDL concentrations to be increased in the metabolic syndrome.4–6 Although the origin of oxLDL is unknown, the vascular wall is considered the main source. In this setting, multiple factors apparently work together to generate oxLDL. The importance of the number and size of LDL particles has been established previously in this context,7,8 but endothelial dysfunction, a local pro-oxidative environment and a proinflammatory state, might also contribute to the generation of oxLDL. These features are often associated with the metabolic syndrome, but whether they coincide and determine oxLDL formation in individuals with the metabolic syndrome is not fully understood.
Over the past decade, the F2-isoprostanes have emerged as a reliable marker of oxidative stress in vivo, and numerous studies have noted increased F2-isoprostane concentrations in a wide range of disease states.9 F2-isoprostanes are stable products formed from the free radical induced peroxidation of phospholipid-bound arachidonic acid, which, in turn, are released from the phospholipids and further metabolized or secreted in the urine.10 Most studies conducted have quantified 8-iso-prostaglandin F2α (PGF2α), which is the major F2-isoprostane formed during lipid peroxidation. The literature concerning F2-isoprostanes and the metabolic syndrome is meager. Type 2 diabetes has been associated consistently with increased concentrations of F2-isoprostanes, but this might be a later phenomenon of the disease.11 A strong link between oxLDL and F2-isoprostanes might be anticipated, but to date, this has not been established. Moreover, the degree of oxidative stress and how dietary habits relate to oxidative stress in the metabolic syndrome is poorly explored. Against this backdrop, we investigated the relationships between oxidative stress, oxLDL, inflammation, and dietary composition in healthy 62- to 64-year-old Swedish men, in whom all components of the metabolic syndrome were characterized in detail.

Methods

Subjects
A total of 289 healthy, 62- to 64-year-old men (mean 63±0.6 years of age) were recruited randomly from a larger population-based cohort of 2039 men living in Stockholm County.12 Recruitment details, exclusion criteria, and clinical procedures have been described.13 The definition of the metabolic syndrome according to the National Cholesterol Education Program/Adult Treatment Panel III (NCEP/ATPIII) was used. Accordingly, the metabolic syndrome was defined as present when a male individual had ≥3 of the following risk factors: plasma glucose ≥6.1 mmol/L; systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg; plasma triacylglycerol >1.7 mmol/L; high-density lipoprotein (HDL) cholesterol (HDL-C) <1.0 mmol/L, and waist circumference >102 cm. Participants were divided into 3 subgroups: (1) subjects with no risk factors, (2) subjects with 1 or 2 risk factors, and (3) subjects fulfilling the criteria for the metabolic syndrome. The ethics committee of Karolinska Institutet approved the study, and all subjects gave informed consent to participate.

Dietary Assessment
The 7-day record14 was a slightly modified, preprinted, optically readable version of a questionnaire used by the Swedish National Food Administration and Statistics Sweden in a national dietary survey in 1989.15 Processing of the data and identification of under-reporters has been described.13 Fatty acids (FAs) quantified in the reported diet were 4:0 to 10:0, 12:0, 14:0, 16:0, 18:0, 16:1, 18:1, Omega-3 FAs 18:3, 20:5, 22:5, 22:6, and Omega-6 FAs 18:2 and 20:4.

Laboratory Procedures
Commercially available ELISAs were used to quantify oxLDL (Merckodia AB), C-reactive protein (CRP; Hemochrom Diagnostica GmbH), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6; R&D Systems Inc.), and insulin and proinsulin (DAKO). Coefficients of variation for the assays were 15% for oxLDL, 11% for CRP, 6% for TNF-α, and 10% for IL-6. Free 8-iso-PGF2α and 15-keto-dihydro-PGF2α (an indicator of cyclooxygenase-mediated inflammation) were determined in 24-hour urine samples by radioimmunoassays as described16,17 (coefficient of variation was 13% for both assays) and corrected for glomerular filtration rate, assessed as equal to the clearance of creatinine per minute and calculated as (urinary creatinine×urinary volume)/serum creatinine×1440). Plasma glucose was measured by a glucose oxidase method (Kodak Ektachem), serum uric acid by uricase method (Johnson & Johnson Vitros), and plasma apolipoprotein B (apoB) by immunoprecipitation (Beckman Coulter, Inc). Cholesterol in very low–density lipoprotein, LDL, and HDL was determined by a combination of preparative ultracentrifugation, precipitation of apoB-containing lipoproteins, and lipid determinations (ABX Diagnostics).18 LDL peak particle size was determined by 3% to 7.5% polyacrylamide gradient gel electrophoresis, staining, and subsequent scanning.13 FAs in serum phospholipids were separated by gas liquid chromatography19 and expressed as the relative molar percentage of the sum of FAs analyzed. The FAs quantified in serum were 14:0, 15:0, 16:0, 17:0, 18:0, 16:1, 18:1, Omega-3 FAs 18:3, 20:5, 22:5, 22:6, and Omega-6 FAs 18:2, 20:3, and 20:4.

Statistical Procedures
Statview (SAS Institute Inc.) software was used for analysis. Skewed data were logarithmically transformed, but arithmetic means (±SD) are presented for ease of understanding. The homeostasis model assessment (HOMA) of insulin sensitivity was derived as described.20 ANOVA was used to test for differences between means and Fisher’s projected least significant difference for post hoc analysis. Outliers, determined as exceeding mean±4×SD were excluded. Because of the high number of univariate analyses performed, the significance level was set to <0.01. The χ2 test was used to test for difference in smoking between groups. A multivariate model with a stepwise forward approach was used with significance levels set to <0.05. Because dietary variables were integrated in the models, multivariate analyses only included individuals identified as dietary non–under-reporters. Variables included in the models had a P value <0.15 in univariate analysis, and correlation coefficients between variables were <0.7.

Results
No subject had overt diabetes mellitus. Twenty-two subjects (8%) fulfilled the criteria for the metabolic syndrome according to the NCEP/ATPIII guidelines, 179 subjects (62%) had ≥1 risk factor (but not the full syndrome), and 88 subjects (30%) had no risk factors. As expected, comparison of these groups revealed significant differences for several metabolic parameters (Table 1). Plasma concentrations of apoB, triacylglycerol, insulin, proinsulin, glucose, and uric acid as well as HOMA index, anthropometric measurements, and blood pressure were positively related to the number of risk factors for the metabolic syndrome. Total and LDL cholesterol (HDL-C) as well as 15-keto-dihydro-PGF2α did not differ significantly between the groups, whereas HDL-C and LDL peak size demonstrated inverse relationships with the number of risk factors. Neither circulating oxLDL nor urinary 8-iso-PGF2α differed between the groups (Figure). Of note, the retrospectively estimated power of this study (calculated using the mean concentration in each group and weighted SD) to detect significant differences for oxLDL and 8-iso-PGF2α was 23% and 11%, respectively. Inflammatory markers CRP and IL-6, but not TNF-α, were increased in individuals with the metabolic syndrome. Dietary analyses showed no significant differences between the groups for the reported intake of total energy, macronutrients, fiber, or biologically important dietary antioxidants. Furthermore, no differences were detected in reported intake of fat subgroups (i.e., saturated, monounsaturated, and polyunsaturated fat; data not shown). Alcohol intake was positively related to the number of risk factors, but the prevalence of smokers did not differ between the groups.
Circulating oxLDL

Univariate correlation analyses showed plasma concentrations of oxLDL to be positively related to apoB, LDL-C, triacylglycerol, proinsulin, and uric acid, whereas a negative relationship was seen to LDL peak size (Table 2). No significant relationships were found between oxLDL and insulin, HOMA index, body mass index, waist circumference, 8-iso-PGF2α, 15-keto-dihydro-PGF2α, or the inflammatory markers (TNF-α, CRP, and IL-6). Furthermore, dietary analysis revealed no relationships between oxLDL and the reported intake of total fat or fat subgroups or most of the biologically important dietary antioxidants (data not shown). However, weak positive relationships were seen between oxLDL and the reported intake of α-tocopherol and selenium (P=0.04 and 0.08, respectively). Of note, selenium was the only dietary variable that entered the multivariate model (see below).

**Table 1. Characteristics of Subjects**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>None (n=88)</th>
<th>One or Two (n=179)</th>
<th>Met Synd (n=22)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoB, g/L</td>
<td>1.1±0.2</td>
<td>1.2±0.3</td>
<td>1.3±0.3</td>
<td>0.0008</td>
</tr>
<tr>
<td>Triacylglycerol, mmol/L</td>
<td>0.97±0.32</td>
<td>1.19±0.58</td>
<td>2.17±0.85</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.7±1.0</td>
<td>6.0±1.0</td>
<td>6.0±1.0</td>
<td>0.18</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.7±0.4</td>
<td>1.7±0.4</td>
<td>1.4±0.3</td>
<td>0.0028†</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.6±0.9</td>
<td>3.7±0.9</td>
<td>3.6±1.1</td>
<td>0.48</td>
</tr>
<tr>
<td>LDL peak, Å</td>
<td>240±4</td>
<td>238±4</td>
<td>234±5</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>31±14</td>
<td>42±24</td>
<td>71±28</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Proinsulin, pmol/L</td>
<td>3.1±3.2</td>
<td>4.0±3.2</td>
<td>9.1±8.3</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.8±0.4</td>
<td>5.1±0.9</td>
<td>6.4±1.9</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.1±0.6</td>
<td>1.6±1.0</td>
<td>3.4±1.9</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.2±2.1</td>
<td>26.1±2.9</td>
<td>30.1±2.9</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>92±7</td>
<td>97±9</td>
<td>108±6</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>120±6</td>
<td>141±14</td>
<td>148±21</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>75±6</td>
<td>83±8</td>
<td>87±10</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Uric acid, mmol/L</td>
<td>0.33±0.06</td>
<td>0.34±0.06</td>
<td>0.39±0.09</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>15-keto-dihydro-PGF2α, nmol·min·L⁻²</td>
<td>15.3±7.6</td>
<td>14.3±6.6</td>
<td>13.9±8.7</td>
<td>0.43</td>
</tr>
<tr>
<td>TNF-α, μg/L</td>
<td>2.2±0.8</td>
<td>2.3±1.3</td>
<td>2.1±0.8</td>
<td>0.54</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.7±2.2</td>
<td>2.0±2.4</td>
<td>3.4±3.7</td>
<td>0.0038†</td>
</tr>
<tr>
<td>IL-6, μg/L</td>
<td>1.4±1.4</td>
<td>1.4±0.8</td>
<td>2.4±3.3</td>
<td>0.0063†</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>17</td>
<td>15</td>
<td>14</td>
<td>0.88</td>
</tr>
<tr>
<td>Dietary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, MJ/day</td>
<td>10.1±1.6</td>
<td>10.3±1.7</td>
<td>10.7±1.3</td>
<td>0.40</td>
</tr>
<tr>
<td>Total fat, E%</td>
<td>35.4±5.3</td>
<td>34.5±5.7</td>
<td>35.6±5.6</td>
<td>0.48</td>
</tr>
<tr>
<td>Carbohydrates, E%</td>
<td>45±5</td>
<td>44±7</td>
<td>42±4</td>
<td>0.21</td>
</tr>
<tr>
<td>Fiber, g/day</td>
<td>21±6</td>
<td>21±6</td>
<td>21±8</td>
<td>0.88</td>
</tr>
<tr>
<td>Alcohol, g/day</td>
<td>15±13</td>
<td>22±19</td>
<td>25±21</td>
<td>0.039</td>
</tr>
<tr>
<td>Selenium, μg/10MJ</td>
<td>39±9</td>
<td>41±10</td>
<td>42±11</td>
<td>0.14</td>
</tr>
<tr>
<td>Folate, μg/10MJ</td>
<td>238±52</td>
<td>228±47</td>
<td>213±54</td>
<td>0.17</td>
</tr>
<tr>
<td>Vitamin C, mg/10MJ</td>
<td>82±42</td>
<td>83±39</td>
<td>75±35</td>
<td>0.60</td>
</tr>
<tr>
<td>α-Tocopherol, mg/10MJ</td>
<td>8.1±1.1</td>
<td>8.2±1.4</td>
<td>8.7±1.8</td>
<td>0.39</td>
</tr>
<tr>
<td>Beta carotene, mg/10MJ</td>
<td>1.8±1.2</td>
<td>1.7±1.2</td>
<td>1.5±1.3</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Mean±SD. †Post hoc tests for the metabolic syndrome group vs “none” and “one or two” groups; P<0.05. E% indicates energy percent. Individuals identified as under-reporting their dietary intake were excluded from dietary analyses.

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

**Urinary 8-ISO-PGF2α**

Univariate correlation analyses revealed no relationships between urinary 8-iso-PGF2α and components of the metabolic syndrome (triacylglycerol, HDL-C, glucose, waist circumference, and blood pressure) or TNF-α, CRP, and IL-6 (Table 2). 8-ISO-PGF2α correlated strongly with 15-keto-dihydro-PGF2α. Furthermore, 8-iso-PGF2α correlated positively with proinsulin and negatively with the relative content of Omega-3 FAs in circulating phospholipids.
Dietary data revealed positive relationships between 8-iso-PGF$_2\alpha$/H9251 and the reported intake of total fat and subgroups of fat, whereas negative relationships were found to the reported intake of fiber, folate, vitamin C, and beta carotene. Correlation analyses were also performed in the different subgroups based on the number of risk factors for the metabolic syndrome. Individuals with the metabolic syndrome demonstrated strong relationships between 8-iso-PGF$_2\alpha$/H9251 and dietary variables (r = 0.67; P = 0.009), polyunsaturated fat (r = 0.68; P = 0.007), Omega-3 FA (r = 0.59; P = 0.03), Omega-6 FA (r = 0.64; P = 0.01), folate (r = -0.59; P = 0.03), and vitamin C (r = -0.49; P = 0.07). Finally, relationships between 15-keto-dihydro-PGF$_2\alpha$/H9251 and parameters in Table 2 were almost identical to those for 8-iso-PGF$_2\alpha$/H9251 (data not shown).

**Multivariate Analyses**

Independent determinants of plasma oxLDL and urinary 8-iso-PGF$_2\alpha$/H9251 were identified using stepwise linear regression analyses (Table 3). The strongest determinants of oxLDL were LDL-C and LDL peak size, but dietary selenium and HDL-C also demonstrated independent relationships with oxLDL. Interestingly, HDL-C entered the model with a positive standardized coefficient, in contrast to its negative relationship with oxLDL in univariate analysis. The model explained 47% of the variation in plasma oxLDL, and introducing 8-iso-PGF$_2\alpha$/H9251 in the model (as a forced variable) increased $R^2$ by only 0.01. The model for 8-iso-PGF$_2\alpha$/H9251 included a wide range of dietary variables, but only dietary folate entered as an independent determinant. Proinsulin and PL-Omega-3 also entered the model, which explained 12% of the variation in urinary 8-iso-PGF$_2\alpha$/H9251.

**Discussion**

The Metabolic Syndrome

Cross-sectional data from this well-characterized cohort of 62- to 64 year-old healthy men have been reported previous-
Grouping individuals according to NCEP/ATPIII criteria identified 22 individuals with the metabolic syndrome. However, these subjects could be considered to have a relatively mild form of the metabolic syndrome because their dyslipidemia was fairly modest (moderately elevated triacylglycerol, high HDL-C, and normal LDL-C). As depicted in Table 1, group comparisons revealed differences not only for parameters included in the definition of the metabolic syndrome but also for other established features of the metabolic syndrome, such as apoB, LDL peak size, uric acid, inflammatory markers, HOMA index, insulin, and proinsulin. Noteworthy are the relationships for proinsulin and uric acid, although not novel as such, these parameters were identified recently as independent predictors of type 2 diabetes and cardiovascular mortality, respectively. Today, hyperproinsulinemia and hyperuricemia are discussed in the context of increased oxidative stress, and the small number of individuals with the metabolic syndrome in the present study should be taken into account when interpreting our results because it reduces our power to identify associations with the metabolic syndrome, but a nonsignificant day-to-day variation in circulating oxLDL concentrations, as shown previously, suggests that we have a robust measurement of this variable.

The level of oxidative stress (urinary 8-iso-PGF$_{2\alpha}$ concentrations) estimated in this study was not significantly related to the metabolic syndrome. This lack of relationship might be explained by the limited power of the study to detect small differences between the groups (as discussed above). Furthermore, the small size of the metabolic syndrome group in combination with the day-to-day intragroup variation in 8-iso-PGF$_{2\alpha}$ concentrations, reported previously to be 12% to 30%, may have led to an underestimation of the relationship between 8-iso-PGF$_{2\alpha}$ and the metabolic syndrome. The literature is scarce concerning 8-iso-PGF$_{2\alpha}$ and the metabolic syndrome. One study (case-control), detected a marked increase of plasma 8-iso-PGF$_{2\alpha}$ in individuals with the metabolic syndrome, but cases were more dyslipidemic and controls were leaner and healthier than the subjects in the present study. Thus, severity of dyslipidemia might influence levels of 8-iso-PGF$_{2\alpha}$. However, the use of plasma samples in the case-control study may have led to overestimation of 8-iso-PGF$_{2\alpha}$ concentrations because of auto-oxidation of lipids, a consequence that might be especially important in the context of high lipids. The use of urine samples (free from lipids) circumvents this potential problem. In the normal state, the mitochondrial electron transport chain is mainly responsible for intracellular ROS generation (predominantly in the form of superoxide), and this formation can be altered by different stimuli. Although increased mitochondrial superoxide generation is well documented as a probable instigator of insulin resistance and β-cell dysfunction, plasma markers of oxidative stress (such as 8-iso-PGF$_{2\alpha}$) do not necessarily have to reflect a modest increase in intracellular ROS. Thus, we speculate that increased formation of 8-iso-PGF$_{2\alpha}$ related to insulin resistance might be a later consequence of prolonged oxidative stress or related to changes in inflammatory responses. This hypothesis is supported by the finding that 8-iso-PGF$_{2\alpha}$ was elevated only in type 2 diabetics with disease duration $\geq$ 7 years but unchanged in newly diagnosed diabetics ($<$ 7 years).

The relationships for inflammatory markers in this study are in agreement with previous observations, associating a chronic low-grade inflammation with the metabolic syndrome. CRP is a well-known predictor of cardiovascular disease, and recently, CRP and IL-6 were shown to predict

### Table 3. Multivariate Analyses for Circulating oxLDL and Urinary 8-Iso-PGF$_{2\alpha}$

<table>
<thead>
<tr>
<th>β</th>
<th>F-to-remove</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxLDL Intercept</td>
<td>3.5</td>
<td>62.2</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.58</td>
<td>118.2</td>
</tr>
<tr>
<td>LDL peak</td>
<td>−0.30</td>
<td>22.3</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.13</td>
<td>4.3</td>
</tr>
<tr>
<td>Dietary selenium</td>
<td>0.10</td>
<td>4.0</td>
</tr>
<tr>
<td>8-Iso-PGF$_{2\alpha}$ Intercept</td>
<td>4.1</td>
<td>1022.8</td>
</tr>
<tr>
<td>Proinsulin</td>
<td>0.20</td>
<td>8.9</td>
</tr>
<tr>
<td>PL-20:4</td>
<td>−0.18</td>
<td>6.3</td>
</tr>
<tr>
<td>Dietary folate</td>
<td>−0.17</td>
<td>5.6</td>
</tr>
</tbody>
</table>

$n=204$. β indicates standardized coefficient. Variables included but not entering the model, for oxLDL: triacylglycerol, proinsulin, 8-iso-PGF$_{2\alpha}$, IL-6, uric acid, and dietary α-tocopherol; for 8-iso-PGF$_{2\alpha}$: LDL-C, uric acid, PL-20:4, and dietary intake total fat, polyunsaturated fat, carbohydrates, fiber, beta carotene, and vitamin C.
the development of type 2 diabetes. Reported relationships between TNF-α and the metabolic syndrome are more inconsistent, sometimes explained by undetectable or highly variable concentrations of TNF-α in the plasma of healthy subjects. Furthermore, we could not detect any significant relationships between inflammatory markers and oxLDL or 8-iso-PGF_2α, suggesting that the inflammatory response might precede observable increases in oxidative stress.

Dietary analysis (performed only in individuals identified as non–under-reporters) revealed a higher alcohol intake in metabolic syndrome individuals, whereas no significant differences were detected for any of the macronutrients or vitamins. The reported alcohol intake in the 2 groups with metabolic risk factors exceeded the Nordic recommendations for alcohol consumption (upper limit 20 g per day), which is in line with findings relating a high alcohol consumption with the incidence of type 2 diabetes.

**Oxidized LDL**

In the present study, oxLDL was captured with the monoclonal antibody mAb-4E6, developed by Holvoet et al, recognizing a conformational epitope in apoB-100 within LDL, which contains aldehyde-substituted lysine residues. Several clinical studies have shown circulating oxLDL measured with mAb-4E6 to be associated with atherosclerotic disease. OxLDL particles are considered minimally modified LDL of unknown origin. Circulating oxLDL derived from leaking plaques has been suggested and cannot be excluded, but most likely, plasma levels of oxLDL are influenced mainly by the degree of local oxidative stress in the arterial wall and susceptibility of LDL to oxidation. Previous studies have shown apoB, LDL-C, and LDL size to be strongly related to circulating oxLDL. These relationships were confirmed in the present study, reflecting the strong influence of the number and size of LDL particles on circulating oxLDL. Unexpectedly, multivariate analysis revealed HDL-C and dietary selenium as positive predictors of oxLDL, which is difficult to interpret given that HDL-C and selenium possess antioxidant properties. Additionally, and somewhat surprisingly, no relationship was found between oxLDL and 8-iso-PGF_2α in univariate analysis. (Of note, the probability of detecting a relevant relationship between these 2 variables \( r > 0.3; P < 0.01 \) in this study would be close to 100%.) Including 8-iso-PGF_2α in the multivariate model for oxLDL (as a forced variable) did not increase the predictive value significantly. This lack of relationship suggests that oxLDL and 8-iso-PGF_2α represent different entities in the process of oxidative modification.

**Oxidative Stress**

No relationships were found between 8-iso-PGF_2α and most metabolic risk factors in our study, possibly because of the mild form of metabolic disturbances in these individuals. The strongest relationship for 8-iso-PGF_2α was to 15-keto-dihydro-PGF_2α, a metabolite and biomarker of cyclooxygenase-catalyzed prostaglandin (PGF_2α) synthesis. Of note, 15-keto-dihydro-PGF_2α had almost identical relationships to those for 8-iso-PGF_2α, implying that these 2 variables are intimately related. The positive relationship between 8-iso-PGF_2α and proinsulin (also seen for oxLDL) might reflect oxidative stress-mediated impaired conversion of proinsulin to insulin, which would be in agreement with the body of data advocating oxidative stress as an instigator of β-cell dysfunction. In fact, proinsulin demonstrated an independent relationship with 8-iso-PGF_2α when entering the multivariate model together with inverse relationships for dietary folate and PL-Omega-3. The latter is in agreement with supplementation studies with Omega-3 FAs, which have resulted in reduced 8-iso-PGF_2α concentrations. However, no relationship between dietary Omega-3 FAs and 8-iso-PGF_2α was detected in the present study. Dietary intake (in energy percent) of total fat and different FAs (irrespective of degree of saturation) was positively related to 8-iso-PGF_2α in univariate analysis. Furthermore, the strong relationships between 8-iso-PGF_2α and fat intake (positively) and dietary antioxidants (negatively) in the subgroup with the metabolic syndrome is intriguing and might be the consequence of these individuals having impaired capacity to handle increased oxidative stress and therefore being particularly sensitive to unfavorable alterations in the diet. Hypothetically, these relationships could reflect a fat-rich, energy-dense diet (irrespective of fat type) in subjects with the metabolic syndrome, which causes a positive energy balance and overloads the mitochondrial electron transport system, leading to increased formation of ROS and 8-iso-PGF_2α.

The defense mechanisms against ROS and other reactive compounds play important roles in preserving tissue structure and function. Several dietary components are involved in this interplay, and certain antioxidants may be important in protection against oxidative stress–mediated tissue damage. In general, varying doses of antioxidant therapy in healthy individuals have shown little effect on 8-iso-PGF_2α concentrations. However, heavy smokers and individuals with metabolic disturbances have shown reduced 8-iso-PGF_2α formation when given different antioxidants. Our results showed 8-iso-PGF_2α to be inversely related to dietary vitamin C, folate, and beta carotene in univariate analysis and to folate in multivariate analysis. These findings might be related to endothelial function. Experimental studies have shown vitamin C, folate, and beta carotene to improve endothelial function, and endothelial dysfunction has, in turn, shown close relationships with oxidative stress (and hence, 8-iso-PGF_2α formation) in several biochemical models. Moreover, proinsulin C-peptide and Omega-3 FAs have also been shown to improve endothelial dysfunction. Therefore, modulation of endothelial function might be the underlying explanation behind the independent relationships seen in the multivariate model.

In conclusion, no increase in plasma oxLDL concentrations and measures of oxidative stress (8-iso-PGF_2α) were detected in these otherwise healthy 63-year-old men with the metabolic syndrome. Furthermore, our results indicate a lack of relationship between oxidative stress and oxLDL, suggesting no direct association between these 2 variables in the oxidative modification process. Finally, dietary data suggests a nutritional influence on lipid peroxidation in individuals with metabolic disturbances.

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References

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