Elevated Gamma-Glutamyltransferase Activity and Perturbed Thiol Profile Are Associated With Features of Metabolic Syndrome

Philippe Giral, Nelly Jacob, Caroline Dourmap, Boris Hansel, Alain Carrié, Eric Bruckert, Xavier Girerd, M. John Chapman

Background—Prospective cohort studies have revealed that plasma γ-glutamyltransferase (GGT) activity exhibits a positive association with coronary artery disease. GGT which is equally elevated in metabolic syndrome (MS), is the major regulator of circulating concentrations of thiol compounds derived from glutathione (GSH) cleavage, ie, cysteine and cysteinyl glycine. We compared the circulating thiol profile in a cohort of patients displaying atherogenic dyslipidemia with and without MS.

Methods and Results—This cross-sectional study involved 1131 dyslipidemic patients in primary prevention of whom 26% presented with MS. GGT activity and plasma cysteinyl-glycine and cysteine concentrations were higher in MS patients; by contrast, levels of GSH were significantly lower (P<10 to 4 for all comparisons versus patients without MS). We compared patient groups on the basis of the number of MS criteria which were concomitantly present. A progressive decrease in glutathione levels in contrast to a progressive increase in both cysteinyl–glycine and cysteine levels, and GGT activity, was observed as a function of the number of MS components in the overall population (P for trend <10⁻⁶).

Conclusion—Dyslipidemic patients exhibiting MS are characterized by elevated GGT activity which is associated with perturbed metabolism of thiol compounds. (Arterioscler Thromb Vasc Biol. 2008;28:587-593)

Key Words: metabolic syndrome • γ-glutamyltransferase • glutathione • cysteine • oxidation

Recent prospective cohort studies have revealed that plasma gamma glutamyltransferase (GGT) activity exhibits a graded positive association with the severity of coronary artery disease (CAD), and equally with cardiovascular morbidity and mortality.¹⁻² GGT is central to the extracellular catabolism of glutathione (GSH), a low molecular weight sulfydryl compound which, as a scavenger of oxygen free radicals, plays a key role in protecting against both intracellular and extracellular oxidative stress.³,⁴ The action of GGT on glutathione results in its cleavage to produce cysteine (cys) and cysteinyl-glycine (Cys-Gly), and as such GGT is the principle regulator of their circulating concentrations.⁴⁻⁵ Cysteine is the most abundant plasma thiol and several reports, including our own, suggest that circulating cysteine levels may be associated with atherosclerosis and CVD.⁶⁻⁷

Our earlier studies demonstrated that the incidence of elevation of circulating hepatic enzymes in a cohort of dyslipidemic patients occurred frequently (27.6%), and in addition revealed that elevation of transaminases and GGT was significantly associated with features of the metabolic syndrome.⁸ Moreover, metabolic syndrome (MS) is typically associated with a subclinical inflammatory state and oxidative stress.¹¹⁻¹³ Indeed, GGT activity is a major determinant of redox state.³,¹²

To evaluate the impact of GGT activity on redox state as a function of the nature and the number of criteria of MS, we determined circulating levels of thiol compounds whose metabolism is under the control of GGT in a cohort of dyslipidemic patients in primary prevention.

Methods

Patients

All patients were referred to our Prevention Center for Dyslipidemia and Cardiovascular Disease by their general practitioner and uniformly exhibited a history of dyslipidemia. All patients were in

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primary prevention and were requested to complete a questionnaire on their medical history, smoking habits, lifestyle and diet, and clinical status. Routine medical examination included weight, height (BMI was calculated as weight/(height)**2), waist circumference, and repetitive blood pressure monitoring of the right arm for at least 30 minutes. Subjects with persistent systolic blood pressure \( \geq 140 \text{ mm Hg} \) or diastolic pressure \( \geq 90 \text{ mm Hg} \) or who were using antihypertensive drugs were considered as hypertensive. All patients underwent an electrocardiographic examination at rest. Patients included in the study displayed either hypercholesterolemia (serum LDL-cholesterol \( \geq 160 \text{ mg/dL} \)), or hypertriglyceridemia (serum triglycerides \( \geq 150 \text{ mg/dL} \)), or low serum HDL cholesterol levels \( \leq 35 \text{ mg/dL} \), or a combination of these features.

Details of alcohol consumption were requested in a sample of patients (n = 782) who were classified as heavy drinkers when estimated alcohol consumption was equal to or above 40 g per day.

Patients with hypothyroidism, malignant disease, severe renal insufficiency, cirrhosis, active liver disease attributable to viral infection (positive serology for virus hepatitis B and C), and those in secondary cardiovascular prevention were excluded. Patients with diabetes were also excluded.

Patients were classified as displaying metabolic syndrome on the basis of the modified Adult Treatment Panel III (ATPIII) criteria. Blood Samples and Analytical Methods

Blood samples were withdrawn by venipuncture between 8:00 and 9:30 AM after an overnight fast. Rigorous conditions for blood sampling and processing are essential for the accurate assessment of plasma thiol levels and were systematically standardized (see references 8–14). Alanine amino transferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transpeptidase (GGT) activities were determined at 37°C according to Klauke et al.15 In a large group of healthy blood donors, 16 upper normal values for men were established as 35, 32, and 42, for ALT, AST, and GGT, respectively, and for women as 26, 27, and 32, respectively.

Table 1. Comparison of Clinical and Biological Variables Between Patients With and Without Metabolic Syndrome (Study Population: 1131 Patients)

<table>
<thead>
<tr>
<th></th>
<th>Metabolic Syndrome</th>
<th>No Metabolic Syndrome</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>295 (26%)</td>
<td>836</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>52 (11)</td>
<td>49 (12)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Sex ratio, M/W</td>
<td>162/133 (55/45)</td>
<td>432/404 (52/48)</td>
<td>0.77</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.1 (4.6)</td>
<td>24.4 (3.4)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>97.7 (11.1)</td>
<td>83.9 (11.0)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>139 (15)</td>
<td>130 (16)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>84 (9)</td>
<td>79 (9)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>125 (42%)</td>
<td>118 (14%)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>69 (24%)</td>
<td>160 (19%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Lipid Lowering treatment, %</td>
<td>131 (44%)</td>
<td>354 (42%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Type of dyslipidemia</td>
<td></td>
<td></td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>Type IIa</td>
<td>545 (65%)</td>
<td>48 (16%)</td>
<td></td>
</tr>
<tr>
<td>Type IIb</td>
<td>206 (25%)</td>
<td>172 (58%)</td>
<td></td>
</tr>
<tr>
<td>Type IV</td>
<td>58 (7%)</td>
<td>68 (23%)</td>
<td></td>
</tr>
<tr>
<td>Hypoalphalipoproteinemia</td>
<td>27 (3%)</td>
<td>7 (3%)</td>
<td></td>
</tr>
<tr>
<td>Heavy drinker, ( \geq 40 \text{ g/d} )</td>
<td>7.6%</td>
<td>4.6%</td>
<td>0.10</td>
</tr>
<tr>
<td>Total Cholesterol, mg/dL</td>
<td>259 (61)</td>
<td>258 (54)</td>
<td>0.86</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>241 (239)</td>
<td>114 (85)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>HDL-Cholesterol, mg/dL</td>
<td>45 (12)</td>
<td>58 (14)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>LDL-Cholesterol, mg/dL§</td>
<td>173 (55)</td>
<td>178 (52)</td>
<td>0.12</td>
</tr>
<tr>
<td>Non HDL-Cholesterol, mg/dL</td>
<td>214 (61)</td>
<td>200 (55)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>5.6 (0.6)</td>
<td>5.0 (0.5)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>82 (17)</td>
<td>80 (15)</td>
<td>0.05</td>
</tr>
<tr>
<td>ALT UI/L</td>
<td>33 (21)</td>
<td>26 (15)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>AST UI/L</td>
<td>35 (21)</td>
<td>26 (15)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>GGT UI/L</td>
<td>46 (51)</td>
<td>28 (30)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>Glutathione, µmol/L</td>
<td>6.2 (2.8)</td>
<td>7.2 (2.9)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>Cysteine, µmol/L</td>
<td>257 (47)</td>
<td>241 (41)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>Cysteinyl-glycine, µmol/L</td>
<td>37.0 (8.8)</td>
<td>34.6 (7.7)</td>
<td>&lt;10–4</td>
</tr>
<tr>
<td>Homocysteine, µmol/L</td>
<td>12.2 (4.5)</td>
<td>11.0 (3.7)</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

§Determined by the Friedewald formula in patients (n = 1092) with triglyceride levels <400 mg/dL.
analyses were carried out with the use of JMP (SAS Institute) for the analysis as a dichotomic variable (0/1: absent/present). Statistical discriminant analyses were used to discriminate among the metabolic syndrome components and to GGT activity and compared patient groups on the basis of the number of metabolic syndrome criteria (respectively 0, 1, 2, 3, 4, and 5 criteria) which were concomitantly present. We performed ANOVA followed by post test for linear trend to determine whether the means of the column increased systematically with progressive number of metabolic syndrome criteria (respectively 0, 1, 2, 3, 4, and 5) which were concomitantly present. We performed ANOVA followed by post test for linear trend to determine whether the means of the column increased systematically with progressive increase in the number of metabolic syndrome components. To discriminate among the metabolic syndrome components and to determine whether their effect on GGT might be dominated by 1 or 2 specific MS components, we performed a multivariate logistic analysis with increase of GGT activity above normal range as the dependant dichotomic variable (0/1: normal/raised). In addition, each of the 5 components in the multivariate analysis was entered in the analysis as a dichotomic variable (0/1: absent/present). Statistical analyses were carried out with the use of JMP (SAS Institute) software.

Results
The major biological and clinical characteristics of the study population (n=1131) separated between patients with (n=295; 26%) or without metabolic syndrome are presented in Table 1. Twenty-one percent of overall patients were hypertensive and 43% were under treatment with lipid-lowering drugs. As expected, HDL-C levels were lower and triglyceride levels elevated in metabolic syndrome patients as compared with patients without this syndrome. However, plasma total cholesterol and LDL-cholesterol levels did not differ between the 2 groups including the subgroup of patients without lipid lowering treatment (LDL-C in untreated patients without metabolic syndrome=183±51 mg/dL, LDL-C in untreated patients with metabolic syndrome=175±54 mg/dL; P=0.11). By contrast, non−HDL-cholesterol levels were higher in metabolic syndrome patients as compared with subjects lacking the syndrome.

The percentage of patients with ALT activities above the upper limit of normal values was 26% in the whole study population and was higher in patients with metabolic syndrome as compared with patients without metabolic syndrome (39% versus 21%, P<10⁻⁶).

Gamma GT activity was correlated with BMI (r=0.20; P<10⁻⁶) and waist circumference (r=0.27; P<10⁻⁶), but the partial correlation coefficient of BMI with GGT was no longer significant (r adj waist=−0.05; ns) although waist circumference remained significantly correlated with GGT (r adj BMI=0.19; P<10⁻⁶).

Among the thiol compounds, subjects presenting metabolic syndrome were distinguished by elevated plasma cysteinyl-glycine and cysteine levels and lower levels of GSH (P<10⁻⁶ for all comparisons as compared with those without the metabolic syndrome). Patients with metabolic syndrome were older than patients without; cysteinyl-glycine, glutathione, and GGT activity were not correlated with age (r=-0.01, -0.07, 0.02, respectively); only cysteine was significantly related to age (r=0.60; P<10⁻⁶). Creatinine levels were slightly higher (+3%) in the metabolic syndrome group (P=0.05). Adjustment of levels of glutathione, cysteinyl-glycine, and cysteine and GGT activity for age and creatinine did not modify mean values or statistical significance. Among the sample of patients in whom alcohol consumption was evaluated, 5.3% were heavy drinkers; the distribution of heavy drinkers between patients with and without metabolic syndrome was not distinct, but heavy drinkers displayed higher levels of GGT (44±39 UI versus 30±34 UI; P=0.01). Transaminase activities were correlated with GGT (r=0.56 and r=0.51, respectively, for AST and ALT activity; P<10⁻⁶ for both) and glutathione (r=0.20 and r=0.25, respectively, for AST and ALT activity; P<10⁻⁶ for both), but the partial correlation coefficients for transaminases and glutathione were no longer significant when adjusted for GGT activity (r adj=0.03 and r adj=0.05, respectively, for AST and ALT activity).

We evaluated the potential relationships of each metabolic syndrome component relative to levels of glutathione, cysteinyl-glycine and cysteine, and to GGT activity (Table 2). Patients who exhibited at least 1 of the 5 components of the metabolic syndrome were distinguished by elevation in cysteinyl-glycine and cysteine levels, as well as GGT activity, but exhibited lower glutathione concentrations as compared with patients lacking any component of the metabolic syndrome. We next compared patient groups on the basis of

Table 2. Relationship of Glutathione (GSH), Cysteinyl-Glycine (Cys-Gly), and Cysteine (Cys) Levels and \( \gamma \)-Glutamyl Transferase Activity (GGT) to Each Metabolic Syndrome (MS) Component

<table>
<thead>
<tr>
<th>MS Component</th>
<th>n (%) Subjects</th>
<th>GSH Mean (CI)</th>
<th>GGT Mean (CI)</th>
<th>Cys-Gly Mean (CI)</th>
<th>Cys Mean (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without any MS component</td>
<td>208 (18%)</td>
<td>7.5 (7.1–7.9)</td>
<td>21 (16–26)</td>
<td>33.6 (32.3–34.7)</td>
<td>228 (223–234)</td>
</tr>
<tr>
<td>Waist circumference ≥102 cm (men)</td>
<td>255 (23%)</td>
<td>6.2 (5.9–6.5)</td>
<td>40 (34–46)</td>
<td>35.9 (34.9–36.9)</td>
<td>259 (253–265)</td>
</tr>
<tr>
<td>≥88 cm (women)</td>
<td>566 (50%)</td>
<td>6.7 (6.5–7.0)</td>
<td>38 (34–41)</td>
<td>36.2 (35.5–36.9)</td>
<td>252 (248–256)</td>
</tr>
<tr>
<td>Blood pressure ≥130/85 mm Hg</td>
<td>293 (26%)</td>
<td>6.9 (6.6–7.3)</td>
<td>35 (31–39)</td>
<td>35.7 (34.7–36.7)</td>
<td>240 (235–244)</td>
</tr>
<tr>
<td>Low HDL-C &lt;40 mg/dL (men)</td>
<td>255 (23%)</td>
<td>6.2 (5.9–6.5)</td>
<td>40 (34–46)</td>
<td>35.9 (34.9–36.9)</td>
<td>259 (253–265)</td>
</tr>
<tr>
<td>&lt;50 mg/dL (women)</td>
<td>583 (52%)</td>
<td>6.7 (6.5–6.9)</td>
<td>39 (35–43)</td>
<td>36.3 (35.6–37.0)</td>
<td>256 (252–259)</td>
</tr>
<tr>
<td>High Triglyceride ≥150 mg/dL</td>
<td>241 (21%)</td>
<td>6.2 (5.8–6.5)</td>
<td>47 (39–54)</td>
<td>36.0 (35.0–37.1)</td>
<td>253 (249–259)</td>
</tr>
<tr>
<td>High Fasting Glucose ≥100 mg/dL (5.5 mmol/L)</td>
<td>293 (26%)</td>
<td>6.7 (6.6–7.3)</td>
<td>35 (31–39)</td>
<td>35.7 (34.7–36.7)</td>
<td>240 (235–244)</td>
</tr>
</tbody>
</table>

Statistical Methodology
Results are expressed as mean (SD) and range for continuous variables and as number and percentage (%) for qualitative variables. We evaluated the relationships of each metabolic syndrome component relative to levels of glutathione, cysteinyl-glycine, and cysteine, and to GGT activity and compared patient groups on the basis of the number of metabolic syndrome criteria (respectively 0, 1, 2, 3, 4, and 5 criteria) which were concomitantly present. We performed ANOVA followed by post test for linear trend to determine whether the means of the column increased systematically with progressive increase in the number of metabolic syndrome components. To discriminate among the metabolic syndrome components and to determine whether their effect on GGT might be dominated by 1 or 2 specific MS components, we performed a multivariate logistic analysis with increase of GGT activity above normal range as the dependant dichotomic variable (0/1: normal/raised). In addition, each of the 5 components in the multivariate analysis was entered in the analysis as a dichotomic variable (0/1: absent/present). Statistical analyses were carried out with the use of JMP (SAS Institute) software.
the number of metabolic syndrome criteria which were concomitantly present (Table 3). As patients with 5 components of metabolic syndrome were rare (n=26), they were combined with patients displaying 4 criteria. Table 3A shows the progressive decrease in glutathione levels and the progressive increase in both cysteinyl–glycine and cysteine levels, and in GGT activity, as a function of the number of metabolic syndrome components in the overall population. The probability value for trend was highly significant for the progressive increase in both cysteinyl–glycine and cysteine levels, and in GGT activity, as a function of the number of metabolic syndrome components in the overall population. Levels, and in GGT activity, as a function of the number of metabolic syndrome components in the overall population. The probability value for trend was highly significant for the progressive increase in both cysteinyl–glycine and cysteine levels, and in GGT activity, as a function of the number of metabolic syndrome components in the overall population. The probability value for trend was highly significant for the progressive increase in both cysteinyl–glycine and cysteine levels, and in GGT activity, as a function of the number of metabolic syndrome components in the overall population. The probability value for trend was highly significant for the progressive increase in both cysteinyl–glycine and cysteine levels, and in GGT activity, as a function of the number of metabolic syndrome components in the overall population. The probability value for trend was highly significant for the progressive increase in both cysteinyl–glycine and cysteine levels, and in GGT activity, as a function of the number of metabolic syndrome components in the overall population.

Table 3. Relationship of Glutathione (GSH), Cysteinyl-Glycine (Cys-Gly), and Cysteine (Cys) Levels and γ-Glutamyl Transferase Activity (GGT) to the Number of Metabolic Syndrome Components (Patients Displaying 4 or 5 Criteria for Metabolic Syndrome Were Merged Into A Single Group)

<table>
<thead>
<tr>
<th>n (%) Subjects</th>
<th>GSH Mean (CI)</th>
<th>GGT Mean (CI)</th>
<th>Cys-Gly Mean (CI)</th>
<th>Cys Mean (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Zero (0) component of M.S.</td>
<td>208 (18%)</td>
<td>7.5 (7.1–7.9)</td>
<td>21 (16–26)</td>
<td>33.7 (32.6–34.8)</td>
</tr>
<tr>
<td>One (1) component of M.S.</td>
<td>300 (29%)</td>
<td>7.4 (7.1–7.7)</td>
<td>27 (23–30)</td>
<td>34.6 (33.7–35.5)</td>
</tr>
<tr>
<td>Two (2) components of M.S.</td>
<td>298 (26%)</td>
<td>6.9 (6.6–7.2)</td>
<td>33 (29–38)</td>
<td>35.3 (34.4–36.2)</td>
</tr>
<tr>
<td>Three (3) components of M.S.</td>
<td>192 (17%)</td>
<td>6.4 (6.0–6.8)</td>
<td>45 (40–51)</td>
<td>36.9 (35.7–38.0)</td>
</tr>
<tr>
<td>Four and five (45) components of M.S.</td>
<td>103 (9%)</td>
<td>6.0 (5.5–6.5)</td>
<td>48 (41–55)</td>
<td>37.2 (35.7–38.8)</td>
</tr>
<tr>
<td>P for trend</td>
<td>&lt;10–4</td>
<td>&lt;10–4</td>
<td>&lt;10–4</td>
<td>&lt;10–4</td>
</tr>
</tbody>
</table>

B. Zero (0) component of M.S. | 132 (20%) | 7.5 (7.0–7.9) | 22 (15–29) | 33.9 (32.5–35.2) | 227 (221–233) |
| One (1) component of M.S. | 183 (28%) | 7.3 (6.8–7.7) | 30 (24–36) | 35.2 (34.1–36.4) | 229 (224–234) |
| Two (2) components of M.S. | 167 (26%) | 6.6 (6.2–7.0) | 35 (29–42) | 35.4 (34.2–36.6) | 235 (230–241) |
| Three (3) components of M.S. | 107 (17%) | 6.1 (5.5–6.6) | 53 (45–61) | 37.3 (35.8–38.8) | 239 (234–246) |
| Four and five (45) components of M.S. | 57 (9%) | 5.9 (5.2–6.7) | 57 (46–68) | 38.1 (36.1–40.2) | 249 (240–256) |
| P for trend | <10–4 | <10–4 | <10–4 | <10–4 |

A. All patients, n=1131; B, patients without lipid-lowering treatment, n=646.

Table 4. Multivariate Logistic Analysis With GGT Activity Above the Normal Range (>32 IU for Women and >42 IU for Men) as the Dependant Dichotomic Variable (0/1: normal/elevated)

A. MS component: Odd ratio [CI] p

<table>
<thead>
<tr>
<th></th>
<th>Odd ratio [CI]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased waist circumference, y/n</td>
<td>1.58 [1.02–2.41]</td>
<td>0.04</td>
</tr>
<tr>
<td>Elevated blood pressure, y/n</td>
<td>1.56 [1.04–2.34]</td>
<td>0.03</td>
</tr>
<tr>
<td>High triglyceride, y/n</td>
<td>3.12 [2.08–4.73]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Low HDL-C, y/n</td>
<td>0.85 [0.55–1.30]</td>
<td>0.45</td>
</tr>
<tr>
<td>High fasting glucose, y/n</td>
<td>1.99 [1.27–3.09]</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Estimate</td>
<td></td>
</tr>
<tr>
<td>Age (continuous)</td>
<td>0.01</td>
<td>0.89</td>
</tr>
</tbody>
</table>

B. MS component: Odd ratio [CI] p

<table>
<thead>
<tr>
<th></th>
<th>Odd ratio [CI]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased waist circumference, y/n</td>
<td>1.88 [1.34–2.63]</td>
<td>0.0002</td>
</tr>
<tr>
<td>Elevated blood pressure, y/n</td>
<td>1.78 [1.30–2.44]</td>
<td>0.0004</td>
</tr>
<tr>
<td>High triglyceride, y/n</td>
<td>2.15 [1.56–2.98]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Low HDL-C, y/n</td>
<td>0.99 [0.70–1.40]</td>
<td>0.97</td>
</tr>
<tr>
<td>High fasting glucose, y/n</td>
<td>1.91 [1.36–2.68]</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Estimate</td>
<td></td>
</tr>
<tr>
<td>Age (continuous)</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Each of the five components of the metabolic syndrome was entered in the analysis as a dichotomic variable (0/1: absent/present); age was also included in the multivariate analysis. A. Patients without lipid-lowering drug treatment (n=646); B, Overall population (n=1131).
ference ≥102 cm for men and ≥88 cm for women, were all significantly associated with increase in GGT.

Discussion

Our cross-sectional study of dyslipidemic patients displaying metabolic syndrome has revealed that significant elevation in GGT activity was closely associated with a potentially prooxidant profile of circulating thiol compounds, as demonstrated by low plasma levels of glutathione and by elevated concentrations of cysteine-glycine and cysteine. Such a profile is indicative of oxidative stress, an emerging CV risk factor. Furthermore, incremental modifications in these parameters occurred in parallel with increase in the number of components of the metabolic syndrome. Finally, multivariate analysis revealed that 4 of the 5 components of the metabolic syndrome were significantly associated with increase in GGT. The well-known strong inverse relationship of triglyceride and HDL-cholesterol levels is a plausible explanation for the absence of significant contribution of low HDL-cholesterol levels to the increase in GGT.

We and others have demonstrated that circulating GGT and transaminases activities are elevated in patients with metabolic syndrome. Moreover several epidemiological studies have demonstrated that GGT is a risk factor for CVD. Recently, Ruttmann et al.21 showed that GGT activity was independently associated with cardiovascular mortality; indeed these findings in a large unselected cohort confirmed an association of progressive increase in GGT activity with overall mortality and cardiovascular events. Additionally, GGT was significantly correlated with several components of the metabolic syndrome, although waist circumference was not measured.22 We observed that BMI was no longer correlated with GGT when adjusted for waist circumference. The association of elevated GGT levels with MS is mediated by insulin resistance and central obesity, and circumference was not measured.22 We observed that BMI was no longer correlated with GGT when adjusted for waist circumference. The association of elevated GGT levels with MS is mediated by insulin resistance and central obesity, and is associated with hepatic steatosis. The association of elevated GGT levels with MS can be related to high insulin resistance and central obesity, and is associated with hepatic steatosis.

In the context of oxidative stress, GGT may contribute to the pathogenesis of atherosclerosis, as its systemic activity is associated with generation of ROS. Furthermore GGT activity has been detected in atheromatous plaques in patients with metabolic syndrome. Moreover, as we excluded patients with positive serology for virus hepatitis B and C and cirrhosis, elevated ALT may be indicative of steatohepatitis.

In our study, patients with positive serology for virus hepatitis B and C and cirrhosis and cysteinyl-glycine, elevated ALT may be indicative of steatohepatitis. With higher GGT activity displayed higher BMI, central adiposity and plasma triglyceride levels, and lower HDL-C concentrations, all of which correspond to key features of the metabolic syndrome. Recently Ashak et al.28 demonstrated a direct relationship between oxidative stress as estimated by determination of plasma levels of glutathione and cysteine and redox state, and early subclinical atherosclerosis as assessed by ultrasound evaluation of carotid intima-media thickness. In this context, it is relevant that glutathione-related antioxidant defenses are decreased in human atherosclerotic plaque tissue.

Circulating levels of cysteinyl-glycine derived from the hydrolysis of GSH were significantly elevated in our patients with metabolic syndrome. Cysteinyl-glycine is a potent reducer of iron in the extracellular milieu and can generate Fe2+ ions, thereby triggering iron-dependent production of reactive oxygen species (ROS), which may in turn initiate oxidation of low-density lipoprotein. Indeed, oxidized LDL particles exert potent proinflammatory activity because of their content of oxidized phospholipids, components which could partially account for the pathophysiological link between elevated GGT activity and atherogenesis.

Levels of cysteine, equally a product of GSH cleavage, were also elevated. In this context, it is relevant that plasma levels of cysteine, a putative biomarker of cardiovascular risk, tend to be elevated in dyslipidemic patients with premature atherosclerosis and coronary heart disease. Indeed cysteine may function as an extracellular regulating factor for thiol disulfide exchange, and may in this way act to maintain adequate redox status. Furthermore, this readily oxidizable compound may give rise to the production of free radical species, thereby promoting oxidative damage of low-density lipoprotein.

Our study prompts new hypotheses regarding the pathophysiological mechanisms which might underlie the role of GGT in promoting vascular disease in metabolic syndrome.55,56 Thus the graded increase in levels of cysteinyl-glycine and cysteine, and in GGT activity, as a function of progressive decrease in GSH concentrations, suggests a relationship between these factors. Indeed, linkage of glutamate to cysteine via the γ-carbon renders GSH refractory to standard proteases and only one enzyme is known to hydrolyze the γ-glutamyl bond in the extracellular milieu. This reaction produces cysteinyl-glycine. Thus metabolism of GSH initiated by GGT may lead to altered redox state and oxidative damage. These GGT-mediated reactions catalyze the oxidation of LDL, and elevated levels of oxidatively-modified LDL are associated with enhanced atherogenicity.54–56 In this context, it is highly relevant that we have previously demonstrated that metabolic syndrome in dyslipidemic patients is intimately associated with elevated systemic levels of 8-isoprostanes, an integrative marker of oxidative stress.

As our study is based on a selected population, it exhibits significant limitations. Indeed, all participants were dyslipidemic; the majority of patients with metabolic syndrome in the general population do however display hypertriglyceridemia or low HDL-cholesterol levels. Thus, we included a large population sample and observed similar
results to those observed in other investigations with respect to GGT activity and the levels of thiol compounds in metabolic syndrome. Our analysis was also limited by the cross-sectional design of the study and we were unable to infer causality. Nonetheless, this observational study can be considered as hypothesis generating. Lastly, in the absence of data on liver fat content, we cannot determine whether increase in GGT activity is directly associated with modification in thiol levels, or whether it reflects higher liver fat content.24 Indeed we cannot exclude the possibility that fatty liver might be directly related to modification of thiol metabolism.

Conclusion
We evaluated components of the redox system in dyslipidemic patients with metabolic syndrome as a function of the number of criteria. Our findings suggest that metabolic syndrome is characterized by elevated GGT activity which is associated with a cascade of abnormalities in the thiol redox system.

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Disclosures
None.

References
17. Altman DG. Practical Statistics for Medical Research. Published in 1991 by Chapman and Hall.
from a glutathione/transferrin system. Free Radic Biol Med. 1998;25:
786–792.
A, Dominici S, Comporti M, Pompella A. Gamma-glutamyl
transpeptidase-dependent iron reduction and LDL oxidation—a potential
34. Paolicchi A, Emdin M, Ghiozeni E, Ciancia E, Passino C, Popoff G,
Pompella A. Human atherosclerotic plaques contain gamma-glutamyl
35. Pastore A, Federici G, Bertini E, Piemonte F. Analysis of glutathione:
implication in redox and detoxification. Clin Chim Acta. 2003;333:
19–39.
36. Paolicchi A, Dominici S, Pieri L, Maellaro E, Pompella A. Glutathione
catabolism as a signaling mechanism. Biochem Pharmacol. 2002;64:
1027–1035.
37. Morrison JA, Jacobsen DW, Sprecher DL, Robinson K, Khoury P,
Daniels SR. Serum glutathione in adolescent males predicts parental
38. Ashfaq S, Abramson JL, Jones DP, Rhodes SD, Weintraub WS, Hooper
WC, Vaccarino V, Harrison DG, Quyyumi AA. The relationship between
plasma levels of oxidized and reduced thiols and early atherosclerosis in
39. Lapenna D, de Gioia S, Ciofani G, Mezzetti A, Ucchino S, Calafiore AM,
Napolitano AM, Di Ilio C, Cuccurullo F. Glutathione-related antioxidant
defenses in human atherosclerotic plaques. Circulation. 1998;97:
1930–1934.
WG. Cysteine/cystine couple is a newly recognized node in the cir-
cuitry for biologic redox signaling and control. FASEB J. 2004;18:
1246–1248.
41. Heinecke JW, Kawamura M, Suzuki L, Chait A. Oxidation of low density
lipoprotein by thiols: superoxide-dependent and -independent mecha-
42. Berliner JA, Watson AD. A role for oxidized phospholipids in athero-
43. Go YM, Jones DP. Intracellular proatherogenic events and cell adhesion
modulated by extracellular thiol/disulfide redox state. Circulation.
44. Nappi AJ, Vass E. Comparative studies of enhanced iron-mediated pro-
duction of hydroxyl radical by glutathione, cysteine, ascorbic acid, and
45. Sakuta H, Suzuki T, Yasuda H, Ito T. Gamma-glutamyl transferase and
metabolic risk factors for cardiovascular disease. Intern Med. 2005;44:
538–541.
46. Ferroni P, Basil S, Falco A, Davi G. Inflammation, insulin resistance,
47. Urakawa H, Katsuki A, Sumida Y, Gabazza EC, Murashima S, Morioka
K, Maruyama N, Kitagawa N, Tanaka T, Hori Y, Nakatani K, Yano Y,
Adachi Y. Oxidative stress is associated with adiposity and insulin
48. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury:
Part I: basic mechanisms and in vivo monitoring of ROS. Circulation.
49. Paolicchi A, Emdin M, Passino C, Lorenzini E, Titta F, Marchi S,
Malvaldi G, Pompella A. Beta-lipoprotein- and LDL-associated serum
gamma-glutamyltransferase in patients with coronary atherosclerosis.
50. Holvoet P, Kritchevsky SB, Tracy RP, Mertens A, Rubin SM, Butler J,
Goodpaster B, Harris TB. The metabolic syndrome, circulating oxidized
LDL, and risk of myocardial infarction in well-functioning elderly people
in the health, aging, and body composition cohort. Diabetes. 2004;53:
1068–1073.
51. Jeppesen J, Hansen TW, Rasmussen S, Ibsen H, Torp-Pedersen C. Meta-
abolic syndrome, low-density lipoprotein cholesterol, and risk of cardio-
vascular disease: a population-based study. Atherosclerosis. 2006;189:
369–374.
PW, D’Agostino RB, Vasan RS, Robins SJ. Increased small low-
density lipoprotein particle number: a prominent feature of the meta-
abolic syndrome in the Framingham Heart Study. Circulation. 2006;
113:20–29.
53. Hansel B, Giraud P, Nobecourt E, Chantepie S, Bruckert E, Chapman MJ,
Kontush A. Metabolic syndrome is associated with elevated oxidative
stress and dysfunctional dense high-density lipoprotein particles dis-
playing impaired antioxidant activity. J Clin Endocrinol Metab. 2004;
89:4963–4971.
Association of the metabolic syndrome with history of myocardial
infarction and stroke in the Third National Health and Nutrition Exami-
DECODE Study Group. Prevalence of the metabolic syndrome and its
relation to all-cause and cardiovascular mortality in non-diabetic European
56. Ginsberg HN, Zhang YL, Hernandez-Ono A. Metabolic syndrome:
focus on dyslipidemia. Obesity (Silver Spring) 2006;14 Suppl
1:41S–49S.
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