Markers Of Oxidative Damage Are Not Elevated In Otherwise Healthy Individuals With The Metabolic Syndrome

1,2Raymond CS Seet, MD; 1Chung-Yung J Lee, PhD; 1,2Erle CH Lim, MD; 2Amy ML Quek, MD; 3Shan-Hong Huang, MSc; 1,2Chin-Meng Khoo, 3Barry Halliwell, DSc

1Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore
2Department of Medicine, National University Hospital, Singapore
3Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore

Address Correspondence to:
Dr Raymond Chee-Seong Seet
Email: raymond_seet@nus.edu.sg

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Objective: The role of oxidative damage in the pathogenesis of metabolic syndrome (MetS) is poorly understood.

Research Design And Methods: A detailed cross-sectional study was performed to assess the relationship between lipid oxidation products, gamma glutamyltransferase (GGT), high-sensitivity C-reactive protein (hs-CRP) and phospholipase activities with respect to the metabolic status in a cohort of otherwise healthy individuals.

Results: A total of 179 individuals (87 males and 92 females) of mean age 43 (standard deviation, 14 years) participated in this study. There were no differences in the levels of plasma F2-isoprostanes, hydroxyeicosatetraenoic acids, cholesterol oxidation products and phospholipase activities in individuals with features of MetS. In multivariate analyses, serum hs-CRP was a consistent independent predictor of MetS.

Conclusions: Minimal changes were observed in multiple markers of oxidative damage in a well-characterized cohort of individuals with features of MetS.
Studies that have examined oxidative damage in healthy individuals with features of metabolic syndrome (MetS) have found conflicting results. In some, markers of oxidative damage have been observed to be minimally altered; whilst in others, they were significantly elevated in those with features of MetS. To resolve these discrepancies in the literature, we conducted a detailed cross-sectional study and measured multiple plasma and urinary markers of oxidative damage in a cohort of healthy individuals. In this study, the MetS was defined using a combination of different definitions based on the modified American Heart Association (AHA)/National Heart, Lung and Blood Institute (NHLBI) criteria and the Homeostatic Model Assessment Insulin Resistance (HOMA-IR) index.

**RESEARCH DESIGN AND METHODS**

We included otherwise healthy individuals with no evidence of vascular diseases in this study. The metabolic syndrome status of individuals was defined using a modified criteria of the AHA/NHLBI and the HOMA-IR index. The blood and urine samples were collected, centrifuged and stored at -80°C before analyses. Lipid profile, high-sensitivity C-reactive protein (hs-CRP), insulin, GGT, PLA2 and PAF-AH activities were measured in serum. Plasma F2-isoprostanes, total HETEs (a mixture of 5(S)-, 12(S)-, 15(S)- and 20-HETE), COPs, allantoin and urinary 8-OHdG were measured by gas chromatography-mass spectroscopy and uric acid was measured in plasma using high performance-liquid chromatography. Different metabolites of urinary F2-isoprostanes were measured, namely 8-iso-F2-isoprostanes, 2,3-dinor-F2-isoprostanes and 2,3-dinor-5,6-dihydro-F2-isoprostanes. Urinary creatinine levels were measured to standardize urinary F2-isoprostanes and 8-OHdG and cholesterol to standardize COPs levels. Power calculations, performed a priori on the primary variables, indicated a minimum sample size of 160 was required for this study. Univariate and multivariate regression analyses were performed, taking into account multiple testing.

**RESULTS**

Of the 179 study participants, 87 were males and 92 females (mean age 43; standard deviation, 14 years). 21 (12%) individuals were obese, 71 (40%) overweight, 69 (38%) normal weight and 9 (5%) underweight. None of the study participants was diagnosed with diabetes mellitus based on their fasting glucose levels. Based on the modified AHA/NHLBI criteria, a total of 14 (8%) individuals fulfilled the criteria for MetS; 66 (37%) had one or two risk components; and 99 (55%) did not have any risk component of MetS. More males have one or two risk components of MetS compared to females (Supplemental Table 1). Significant correlation was observed between the number of risk components of MetS with respect to the HOMA-IR index (r= 0.699, p<0.001). Although there were no differences in age, diastolic blood pressure, fasting serum insulin, HOMA-IR index and the number of risk components of MetS, several differences in hemodynamic and metabolic parameters were observed between gender. Males had higher levels of systolic blood pressure, fasting serum glucose, triglycerides and body mass index, while females had higher levels of high-density lipoproteins (Supplemental
To take into account these differences, gender-specific analyses were subsequently performed. There were no significant differences in the levels of the esterified and free forms of plasma F$_2$-isoprostanes, total HETEs, 7β-, 24- and 27-hydroxycholesterol, plasma allantoin, serum PLA$_2$ and PAFAH activities, urinary 8-OHdG (a marker of oxidative damage to DNA and the DNA precursor pool that is known to be elevated in diabetic subjects) and urinary total F$_2$-isoprostanes according to the different risk categories of MetS in males and females. This conclusion was not changed after values were corrected for their precursors (arachidonic acid or cholesterol) (Supplemental Tables 2-4).

On the other hand, serum hs-CRP correlated significantly with the number of risk components of MetS and the HOMA-IR index in both males and females (p-trend<0.001). In females, plasma uric acid and serum GGT were increased in individuals with a higher number of risk components of MetS and the HOMA-IR index; while in males, plasma 7α-hydroxycholesterol correlated significantly with the HOMA-IR index (but not with the number of the risk components of MetS).

To identify predictors of MetS, significant variables were included in a stepwise multivariable model (Table 1). We observed serum hs-CRP to be a consistent predictor of MetS using the 2 different criteria in both males and females. Using the modified AHA/NHLBI criteria, serum hs-CRP accounted for approximately 19% of the variation in the number of risk components of MetS in males, while serum hs-CRP and GGT explained approximately 24% variation in females.

**CONCLUSIONS**

The levels of oxidation products of arachidonic acid (F$_2$-isoprostanes and total HETEs), phospholipase activities (PLA$_2$ and PAFAH), certain cholesterol oxidation products (such as 24- and 27-hydroxycholesterol), 8-OHdG and allantoin (a product of oxidative damage to uric acid) were unchanged across the different risk categories of MetS.

The temporal involvement of oxidative damage in the pathological processes of MetS is poorly understood. In a study among Indian Mauritians with impaired glucose metabolism, plasma F$_2$-isoprostanes were observed to be increased during the initial pre-diabetic and early diabetic states, which led to the suggestion that oxidative damage may precede the development of diabetes mellitus in healthy individuals. In another study that examined oxidative damage in type 2 diabetes mellitus, the levels of urinary F$_2$-isoprostanes were found to be elevated only in those with at least seven years of disease which indicates that oxidative damage is possibly a late consequent of diabetes mellitus. In the present cohort, we found serum hs-CRP (but not markers of oxidative damage) to correlate closely with the number of risk components of MetS and the HOMA-IR index. These data appear to support previous suggestions that low-grade inflammatory changes may occur early prior to the development of cardiovascular diseases.

In this study, we observed gender-specific differences in the correlation of certain markers of oxidative damage and the risk categories of MetS. For example, plasma uric acid and serum GGT correlated significantly with features of MetS in females, while plasma 7α-hydroxycholesterol correlated...
significantly with the HOMA-IR index in males. The reasons for these observations are not known, although gender-specific factors such as differences in the hormonal and metabolic profiles may (at least in part) provide explanations to these findings.

To conclude, minimal changes were observed in multiple markers of oxidative damage in a well-characterized cohort of individuals with features of MetS.

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REFERENCES


Table 1. Multivariable correlates of the number of risk components of Metabolic Syndrome and the Homeostasis Model Assessment Insulin Resistance (HOMA-IR) index

<table>
<thead>
<tr>
<th>Number of risk components of metabolic syndrome (modified AHA/NHLBI criteria)</th>
<th>Regression Coefficient</th>
<th>P-value</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum hs-CRP</td>
<td>0.451</td>
<td>&lt;0.001</td>
<td>0.189</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum hs-CRP</td>
<td>3.826</td>
<td>&lt;0.001</td>
<td>0.243</td>
</tr>
<tr>
<td>Serum GGT</td>
<td>2.584</td>
<td>0.012</td>
<td></td>
</tr>
</tbody>
</table>

| HOMA-IR index                                |                        |         |             |
| **Males**                                    |                        |         | 0.262       |
| Serum hs-CRP                                 | 0.439                  | <0.001  |             |
| Urinary 2,3-dinor-F₂-IsoPs/creatinine         | -0.219                 | 0.036   |             |
| **Females**                                  |                        |         | 0.148       |
| Serum hs-CRP                                 | 0.233                  | 0.027   |             |
| Plasma 7β-hydroxycholesterol                 | 0.316                  | 0.003   |             |

Abbreviations: hs-CRP=high sensitivity C-reactive protein, GGT=gamma glutamyltransferase, AHA/NHLBI=C-reactive protein, GGT=gamma glutamyltransferase, AHA/NHLBI=American Heart Association on (AHA)/National Heart, Lung and Blood Institute criteria