Markers of Oxidative Damage Are Not Elevated in Otherwise Healthy Individuals With the Metabolic Syndrome

RAYMOND C.-S. SEET, MD[1,2]
CHUNG-YUNG J. LEE, PHD[1]
ERLE C.H. LIM, MD[1,2]
AMY M.L. QUEK, MD[2]
SHAN-HONG HUANG, MSC[3]
CHIN-MENG KHOO, MD[1,2]
BARRY HALLIWELL, DSC[3]

OBJECTIVE — The role of oxidative damage in the pathogenesis of metabolic syndrome is poorly understood.

RESEARCH DESIGN AND METHODS — A detailed cross-sectional study was performed to assess the relationship between lipid oxidation products, γ-glutamyltransferase, 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 men and 92 women) aged 43 ± 14 years (mean ± SD) participated in this study. There were no differences in the levels of plasma F_2-isoprostanes, hydroxyeicosatetraenoic acids, cholesterol oxidation products, and phospholipase activities in individuals with features of metabolic syndrome. In multivariate analyses, serum hs-CRP was a consistent independent predictor of metabolic syndrome.

CONCLUSIONS — Minimal changes were observed in multiple markers of oxidative damage in a well-characterized cohort of individuals with features of metabolic syndrome.

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Studies that have examined oxidative damage in healthy individuals with features of metabolic syndrome have shown conflicting results (1–6). In some, markers of oxidative damage have been observed to be minimally altered (1–3), whereas in others, they were significantly elevated in those with features of metabolic syndrome (4–6). 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 metabolic syndrome was defined using a combination of different definitions based on the modified American Heart Association (AHA)/National Heart, Lung, and Blood Institute (NHLBI) criteria (7) and the homeostasis model assessment of insulin resistance (HOMA-IR) index (8).

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 modified criteria of the AHA/NHLBI (7) and the HOMA-IR index (8). 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, γ-glutamyltransferase (GGT), phospholipase A_2, and platelet-activating factor acetylhydrolase (PAF-AH) activities were measured in serum. Plasma F_2-isoprostanes, total hydroxyeicosatetraenoic acid (HETEs) [a mixture of 5(*S*)-, 12(*S*)-, 15(*S*)-, and 20-HETE], cholesterol oxidation products, allantoin, and urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) were measured by gas chromatography–mass spectroscopy (9–12), and uric acid was measured in plasma using high-performance liquid chromatography. Different metabolites of urinary F_2-isoprostanes were measured, namely 8-iso-F_2-isoprostanes, 2,3-dinor-F_2-isoprostanes, and 2,3-dinor-5,6-dihydro-F_2-isoprostanes (10). Urinary creatinine levels were measured to standardize urinary F_2-isoprostanes and 8-OHdG and cholesterol to standardize cholesterol oxidation product levels. Power calculations, performed a priori on the primary variables, indicated that a minimum sample size of 160 was required for this study. Univariate and multivariate regression analyses were performed, taking into account multiple *t* testing.

RESULTS

— Of the 179 study participants, 87 were men and 92 were women (aged 43 ± 14 years [mean ± SD]). Of these, 21 (12%) were obese, 71 (40%) were overweight, 78 (44%) were normal weight, and 9 (5%) were underweight. None of the study participants had diabetes based on their fasting glucose levels. Based on the modified AHA/NHLBI criteria, a total of 14 (8%) individuals fulfilled the criteria for metabolic syndrome; 66 (37%) had one or two risk components; and 99 (55%) did not have any risk component of metabolic syndrome. More men had one or two risk components of metabolic syndrome than women (supplementary Table 1, available in an online appendix at http://care.diabetesjournals.org/cgi/content/full/dc09-2124/DC1). A significant correlation was observed between the number of risk components of metabolic syndrome 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 metabolic syndrome, several differences in hemodynamic and metabolic parameters were observed between the sexes. Men had

From the [1]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; the [2]Department of Medicine, National University Hospital, Singapore; and the [3]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.
Corresponding author: Raymond C.-S. Seet, raymond_seet@nus.edu.sg.
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Table 1—*Multivariable correlates of the number of risk components of metabolic syndrome and the HOMA-IR index*

	Regression coefficient	*P* value	Adjusted R^2
No. risk components of metabolic syndrome (modified AHA/NHLBI criteria)			
Men			0.189
Serum hs-CRP	0.451	<0.001	
Women			0.243
Serum hs-CRP	3.826	<0.001	
Serum GGT	2.584	0.012	
HOMA-IR index			
Men			0.262
Serum hs-CRP	0.439	<0.001	
Urinary 2,3-dinor-F_2-isoprostanes/creatinine	−0.219	0.036	
Women			0.148
Serum hs-CRP	0.233	0.027	
Plasma 7β-hydroxycholesterol	0.316	0.003	

higher levels of systolic blood pressure, fasting serum glucose, triglycerides, and BMI, whereas women had higher levels of HDLs (supplementary Table 1). To take into account these differences, sex-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 PAF-AH 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) (13); and urinary total F_2-isoprostanes according to the different risk categories of metabolic syndrome in men and women. This conclusion was not changed after values were corrected for their precursors (arachidonic acid or cholesterol) (supplementary Tables 2–4, available in an online appendix). On the other hand, serum hs-CRP correlated significantly with the number of risk components of metabolic syndrome and the HOMA-IR index in both men and women ($P_{trend}<0.001$). In women, plasma uric acid and serum GGT were increased in individuals with a higher number of risk components of metabolic syndrome and the HOMA-IR index, whereas in men, plasma 7α-hydroxycholesterol correlated significantly with the HOMA-IR index (but not with the number of the risk components of metabolic syndrome).

To identify predictors of metabolic syndrome, significant variables were included in a stepwise multivariable model (Table 1). We observed serum hs-CRP to be a consistent predictor of metabolic syndrome using the two different criteria in both men and women. With use of the modified AHA/NHLBI criteria, serum hs-CRP accounted for ~19% of the variation in the number of risk components of metabolic syndrome in men, whereas serum hs-CRP and GGT explained ~24% variation in women.

CONCLUSIONS — The levels of oxidation products of arachidonic acid (F_2-isoprostanes and total HETEs), phospholipase activities (PLA_2 and PAF-AH), 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 metabolic syndrome.

The temporal involvement of oxidative damage in the pathological processes of metabolic syndrome 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 prediabetic and early diabetic states, which led to the suggestion that oxidative damage may precede the development of diabetes in healthy individuals (6). In another study that examined oxidative damage in type 2 diabetes, the levels of urinary F_2-isoprostanes were found to be elevated only in those with at least 7 years of disease (14), which indicates that oxidative damage is possibly a late consequent of diabetes. 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 metabolic syndrome and the HOMA-IR index. These data seem to support previous suggestions that low-grade inflammatory changes may occur early before the development of cardiovascular diseases (14).

In this study, we observed sex-specific differences in the correlation of certain markers of oxidative damage and the risk categories of metabolic syndrome. For example, plasma uric acid and serum GGT correlated significantly with features of metabolic syndrome in women, whereas plasma 7α-hydroxycholesterol (15) correlated significantly with the HOMA-IR index in men. The reasons for these observations are not known, although sex-specific factors such as the differences in the hormonal and metabolic profiles may (at least in part) provide explanations for these findings.

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

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