Elevated Interleukin-18 Levels Are Associated With the Metabolic Syndrome Independent of Obesity and Insulin Resistance

Joseph Hung, Brendan M. McQuillan, Caroline M.L. Chapman, Peter L. Thompson, John P. Beilby

Objective—Activated innate immunity is thought to be involved in the pathogenesis of metabolic syndrome and type 2 diabetes. Interleukin-18 (IL-18) is a pleiotropic proinflammatory cytokine with important regulatory functions in the innate immune response. We sought to determine whether an elevated IL-18 concentration was a risk predictor for metabolic syndrome in a community population independent of obesity and hyperinsulinemia.

Methods and Results—A representative general population, aged 27 to 77 years, without clinical diabetes was studied for clinical and biochemical risk factors for metabolic syndrome. Serum IL-18 concentration measured in 955 subjects correlated with metabolic syndrome traits including body mass index (BMI), waist circumference, triglyceride, high-density lipoprotein (inversely), and fasting glucose and insulin levels (all \( P < 0.001 \)). Mean IL-18 levels rose progressively with the increasing number of metabolic risk factors (ANOVA \( P < 0.001 \)). After adjusting for age, gender, BMI, and insulin levels, increasing IL-18 tertiles were associated with an odds ratio for metabolic syndrome of 1.0, 1.42, and 2.28, respectively (\( P \) trend =0.007). The graded risk relation was even stronger in nonobese subjects and not attenuated when adjusted for C-reactive protein and IL-6 levels.

Conclusion—Our findings support the hypothesis that activation of IL-18 is involved in the pathogenesis of the metabolic syndrome.

Key Words: IL-18 ■ metabolic syndrome ■ obesity ■ insulin resistance ■ inflammatory mediators

Metabolic syndrome is a heterogeneous condition characterized by visceral adiposity, dyslipidemia, hypertension, and insulin resistance.\(^1\),\(^2\) The metabolic syndrome with its clustering of metabolic and atherosclerotic risk factors is a strong determinant of type 2 diabetes and cardiovascular disease (CVD).\(^3\)–\(^5\) Obesity and insulin resistance are considered central to the pathophysiology of this metabolic and cardiovascular syndrome.\(^6\),\(^7\) Recently, activated innate immunity and chronic inflammation have also been causally implicated and may represent a potential link between metabolic syndrome, diabetes, and atherosclerosis.\(^8\)–\(^10\)

Several cross-sectional studies have shown that acute-phase reactants such as C-reactive protein (CRP) and cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-\(\alpha\) associate with features of the metabolic syndrome such as body mass index (BMI)/waist circumference, measures of insulin resistance/plasma insulin concentration, hypertension, and dyslipidemia.\(^1\)–\(^10\) However, it is uncertain whether the association of inflammatory markers with metabolic syndrome is independent of measures of obesity and insulin resistance when they are included in a risk prediction model.\(^17\)–\(^19\)

IL-18, a recently described member of the IL-1 cytokine superfamily, is now recognized as an important regulator of innate and acquired immune responses.\(^20\),\(^21\) It is a potent proinflammatory cytokine, and a role in plaque destabilization has been suggested.\(^22\) Prospective studies have shown an association of circulating IL-18 levels with cardiovascular death in patients with coronary artery disease and with coronary events in apparently healthy men.\(^23\),\(^24\)

There is some evidence that IL-18 levels may be linked with metabolic risk factors, although the role of IL-18 in the metabolic syndrome has not been specifically studied. IL-18 levels have been associated with adiposity and insulin resistance in obese premenopausal women.\(^25\),\(^26\) IL-18 concentrations are increased by acute hyperglycemia in humans through an oxidative mechanism,\(^27\) and patients with type 2 diabetes have higher IL-18 levels than matched nondiabetic subjects.\(^28\),\(^29\)

The purpose of this study was to determine whether circulating IL-18 levels were associated with features of the metabolic syndrome in a large cross-sectional community-based population. In particular, we sought to establish whether circulating IL-18, as well as IL-6 and CRP, were risk...
predictors of the metabolic syndrome independent of obesity and insulin resistance.

Methods

Study Population

The selection criteria and study design of the community-based Carotid Ultrasound Disease Assessment Study (CUDAS) have been detailed previously. In brief, this was a random electoral roll sample of 1111 subjects (558 men, 553 women) aged 27 to 77 years from Perth, Western Australia, who were assessed for cardiovascular risk factors and had carotid B-mode ultrasound performed. This present study sample was confined to the 960 subjects without a clinical history of diabetes and who had a fasting serum glucose measured. Subjects also had available measurements of fasting serum insulin (n = 959), high-sensitive (hs) CRP (n = 944), IL-6 (n = 959), and IL-18 (n = 238). A self-administered questionnaire was used to record a clinical history of smoking, hypertension, or diabetes. Anthropomorphic measurements and the lower of 2 resting blood pressures were recorded. BMI was calculated as weight (in kilograms)/height (in meters). The study protocol was approved by the institutional ethics committee of the University of Western Australia. Written informed consent was obtained from all study participants.

As detailed in the 2001 National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) report, the metabolic syndrome was defined as ≥3 of the following characteristics: abdominal obesity as measured by waist circumference of >102 cm in men and >88 cm in women; hypertriglyceridemia ≥150 mg/dL (≥1.69 mmol/L); low high-density lipoprotein (HDL) cholesterol <40 mg/dL (<1.0 mmol/L) in men and ≤50 mg/dL (<1.3 mmol/L) in women; high blood pressure ≥130 mm Hg systolic or ≥85 mm Hg diastolic or current use of antihypertensive drugs; and high fasting glucose ≥110 mg/dL (≥6.1 mmol/L).

Biochemical Analysis

A fasting blood sample was obtained from each subject. Serum IL–18 was measured by a commercially available ELISA method (MBL Co. Ltd.) as described previously. The within-run coefficient of variation (CV) was 5.4% at a mean value of 400 μg/L (28 samples); between-run CV was 8.2% at 298 μg/L (9 samples) and 7.8% at 496 μg/L (9 samples). Serum IL–6 was measured using an ELISA (Quantikine HS; R & D Systems), with an assay range of 0.38 to 10.0 ng/mL. Serum hs-CRP was measured by a microparticle turbidity assay with a range of 0.1 to 21.0 mg/mL. Insulin was measured as mU/L on a Tosoh AIA-600 immunoassay analyzer using a 2-site immunoenzymometric assay. The within-run CV was 4.0% at a mean value of 13.8 mU/L (80 samples); between-run CV was 5.6% at 14.3 mU/L (6 samples) and 5.6% at 20.0 mU/L (6 samples). Total cholesterol, HDL cholesterol, and triglyceride levels were determined enzymatically with a Hitachi 747 autoanalyzer.

Statistical Analysis

Outcome variable of the association analyses was metabolic syndrome as defined by NCEP ATP III criteria. The principal explanatory variables were the inflammatory markers IL–18, IL–6, and hs-CRP. Covariates included in regression analyses were age, gender, BMI, fasting insulin, low-density lipoprotein cholesterol, and smoking history (pack years). Serum levels of IL–18, IL–6, and hs-CRP were not normally distributed and therefore log (base e) transformed. Geometric means and 95% CIs are given for these variables. Continuous variables were then compared using ANOVA. Categorical variables were compared by χ² test. Statistical significance was taken as P<0.05.

Nonparametric Spearman rank correlations were used to describe the univariate association of inflammatory markers and metabolic risk factors. Forward and backward stepwise multiple logistic regression analysis was used to determine independent predictors of metabolic syndrome and calculate odds ratios and 95% CI for each risk variable. The best fit logistic regression model was found by exhaustive search of all univariate correlates of metabolic syndrome other than those variables that were used to define metabolic syndrome (Table 1). BMI, fasting insulin, IL–18, IL–6, and hs-CRP levels were entered as tertile categories in the logistic model. As recommended, subjects with CRP >10 mg/L were excluded from analysis. Subjects were defined as nonobese if they had a BMI <30 kg/m². Potential interaction effects on metabolic syndrome between the inflammatory markers and age, sex, or BMI were explored by

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Metabolic Syndrome Risk Factors</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Age, years</td>
<td>n=243</td>
<td>n=306</td>
</tr>
<tr>
<td>Male, %</td>
<td>45.8 (43.3–47.3)</td>
<td>54.5 (53.1–55.8)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.5 (23.0–23.9)</td>
<td>25.1 (24.7–25.4)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>75.7 (74.4–77.0)</td>
<td>81.6 (80.5–82.8)</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>0.9 (0.8–0.9)</td>
<td>1.0 (0.9–1.1)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.56 (1.52–1.60)</td>
<td>1.43 (1.40–1.47)</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol, mmol/L</td>
<td>3.4 (3.3–3.5)</td>
<td>3.6 (3.5–3.7)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>111 (110–114)</td>
<td>130 (126–132)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>72 (71–73)</td>
<td>81 (80–82)</td>
</tr>
<tr>
<td>Smoking, pack years</td>
<td>7.7 (5.0–10.3)</td>
<td>10.4 (8.0–12.8)</td>
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<tr>
<td>Glucose, mmol/L</td>
<td>5.2 (5.1–5.3)</td>
<td>5.5 (5.4–5.6)</td>
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<tr>
<td>Insulin, mU/L</td>
<td>4.2 (3.5–4.8)</td>
<td>5.1 (4.5–5.6)</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>1.14 (1.00–1.30)</td>
<td>1.57 (1.39–1.77)</td>
</tr>
<tr>
<td>IL–6, μg/L</td>
<td>3.08 (2.92–3.26)</td>
<td>3.47 (3.31–3.65)</td>
</tr>
<tr>
<td>IL–18, μg/L</td>
<td>255 (243–268)</td>
<td>279 (267–292)</td>
</tr>
</tbody>
</table>

Data are mean or geometric mean and 95% CIs.

*Criteria for metabolic syndrome according to NCEP ATP III Report 2001 (see Methods).
including the interaction term, hs-CRP, IL-6, or IL-18 by age, sex, or BMI in the multivariate analysis. SPSS version 10.1 for Windows was used in analysis.

### Results

#### Clinical Characteristics

Table 1 shows the clinical and biochemical characteristics of the study population according to the number of ATP III criteria for metabolic syndrome. On the basis of ≥3 criteria being present, 173 subjects (18% of the population) were defined as having the metabolic syndrome. The population was largely asymptomatic, with a history of coronary heart disease or stroke present in only 7.3% (n=70), use of cholesterol-lowering drugs in 6.1% (n=59), and antihypertensive drugs in 14.9% (n=143). As expected, there was a positive association of age, male gender, BMI, waist circumference, blood pressure, triglyceride, glucose, and insulin levels and an inverse association of HDL level with the number of metabolic risk factors (all ANOVA P<0.001). There was also a positive association of smoking exposure with metabolic syndrome. Geometric mean concentrations of hs-CRP, IL-6, and IL-18 increased progressively with the escalating number of metabolic risk factors (all ANOVA P<0.001).

### Correlation Between Metabolic Syndrome Traits and Inflammatory Markers

Table 2 shows Spearman’s rank correlation coefficients (r_s) between individual metabolic syndrome traits and fasting insulin, hs-CRP, IL-6, and IL-18 concentrations. Predictably, individual components of the metabolic syndrome were associated with each other (all P<0.001). In particular, BMI and waist circumference were highly correlated measures of obesity (r_s=0.80). Fasting insulin showed moderate correlations with BMI, waist circumference, triglyceride, glucose, and insulin levels (all P<0.001) and showed a stronger association than fasting glucose with most of the metabolic traits (Table 2).

Levels of IL-18 were correlated with hs-CRP (r_s=0.18) and IL-6 (r_s=0.22; both P<0.001). On the whole, IL-18, IL-6, and hs-CRP concentrations showed moderate associations with BMI, waist circumference, triglyceride, HDL (inversely), blood pressure, and insulin levels (Table 2). IL-18 correlated more strongly with waist circumference (r_s=0.39) and HDL (r_s=−0.31) than with other metabolic traits and was weakly associated with age (r_s=0.10). There was no significant difference between males and females in the association of IL-18 with metabolic traits.

### Predictors of Metabolic Syndrome

Multivariate analysis established that age, male gender, BMI, and fasting insulin levels were independent predictors of the metabolic syndrome (Table 3). For example, subjects in the top compared with bottom tertiles of BMI and insulin level had an adjusted odds ratio for metabolic syndrome of 6.75 and 7.11, respectively (both P<0.001).
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Table 4 shows the multivariate odds ratios for metabolic syndrome associated with tertiles of hs-CRP, IL-6, and IL-18. After adjusting for age and gender, the top compared with bottom tertiles of hs-CRP and IL-6 were associated with a 2- to 3-fold increased odds ratio for metabolic syndrome (P<0.001). However, there was no longer a significant association after BMI and insulin were added to the model (Table 4). Further exploratory analysis indicated a significant interaction between hs-CRP and BMI (P for interaction=0.007) but not between hs-CRP and age or sex. When analysis was confined to nonobese subjects, the age, gender, and insulin-adjusted odds ratios for metabolic syndrome were 1.00, 1.55, and 2.32 for increasing tertiles of hs-CRP (P trend=0.036) and 1.0, 1.31, and 2.03 for increasing tertiles of IL-6 (P trend=0.08).

In comparison, IL-18 remained an independent risk predictor for metabolic syndrome even when adjusted for age, sex, BMI, and insulin level (Table 4). Increasing tertiles of IL-18 were associated with adjusted odds ratios for metabolic syndrome of 1.0, 1.42, and 2.38, respectively (P trend=0.007). A significant interaction between IL-18 and BMI was also indicated on exploratory analysis (P for interaction=0.03), and when the population was stratified by BMI, a >3-fold odds ratio for metabolic syndrome was found for nonobese subjects who had an IL-18 concentration in the top versus bottom tertile (P<0.001; Table 4). The addition of hs-CRP and IL-6 in the multivariate model for the whole cohort did not attenuate the relationship between IL-18 levels and metabolic syndrome with odds ratios of 1.0, 1.46, and 2.47 for increasing tertiles of IL-18 (P trend=0.004).

Discussion

We report for the first time that elevated IL-18 levels were an independent risk predictor for the metabolic syndrome in the absence of diabetes history in a large community population sample. The association was independent of the major determinants of metabolic syndrome, namely obesity and insulin resistance. Further adjustment for hs-CRP and IL-6 levels did not attenuate the relationship between IL-18 and metabolic syndrome. Obesity confounded the relationship between hs-CRP and IL-6 levels and metabolic syndrome. However, elevated hs-CRP and IL-6 levels were independent risk predictors for the metabolic syndrome in nonobese subjects.

Inflammation and activated innate immunity are thought to play an important role in the development of atherosclerosis and diabetes and may represent a unifying link between metabolic syndrome, type 2 diabetes, and CVD. Consistent with previous cross-sectional studies, we found that inflammatory markers clustered with metabolic syndrome traits. We also demonstrated that circulating levels of IL-18, IL-6, and hs-CRP increased progressively with the escalating number of metabolic traits. The inflammatory markers also showed a positive association with fasting insulin level, a reasonable surrogate measure of insulin resistance.

Although elevated levels of these inflammatory markers may indicate that chronic inflammation is causally involved in the pathway of metabolic syndrome, they may also simply be markers of associated obesity, insulin resistance, or other metabolic risk traits. In particular, IL-6 and hs-CRP are associated with visceral adiposity, because ~30% of circulating IL-6 is derived from human adipose tissue, and hepatic synthesis of CRP is largely regulated by IL-6. In the present study, their risk relation to metabolic syndrome was largely attenuated after adjustment for BMI in the multivariate model (Table 4). Others have found that CRP concentrations can be related to insulin resistance independent of obesity but because insulin resistance is considered a primary defect of metabolic syndrome, we also included insulin level as a covariate in the multivariate model (Tables 3 and 4).

Exploratory analysis in this study did suggest an interaction between BMI and CRP on the likelihood of metabolic syndrome. Stratification of the population sample by BMI

| Variable | Age and Sex-Adjusted | | Age, Sex, BMI, and Insulin Adjusted | | Age, Sex and Insulin Adjusted in Nonobese Subjects* |
|----------|---------------------|-------------------|-----------------------------|-----------------------------|
|          | Odds ratio (95% CI) | P Value           | Odds ratio (95% CI)         | P Value                     |
|          | n=910               |                   | n=910                       |                               |
| n=765    |                     |                   |                             |                               |
| hs-CRP, mg/L |                  |                   |                             |                               |
| <1.01    | 1.00               |                   |                             |                               |
| 1.01–2.40| 1.41 (0.86–2.33)   | 0.17              | 0.94 (0.53–1.66)            | 0.84                        |
| >2.40    | 2.93 (1.85–4.63)   | <0.001            | 1.11 (0.64–1.93)            | 0.70                        |
|          | <0.001†            |                   |                             |                               |
| IL-6, μg/L |                  |                   |                             |                               |
| <2.94    | 1.00               |                   |                             |                               |
| 2.94–3.90| 1.53 (0.93–2.49)   | 0.09              | 1.29 (0.74–2.25)            | 0.37                        |
| >3.90    | 2.58 (1.60–4.16)   | <0.001            | 1.46 (0.84–2.56)            | 0.18                        |
|          | <0.001†            |                   |                             |                               |
| IL-18, μg/L |                |                   |                             |                               |
| <251     | 1.00               |                   |                             |                               |
| 251–356  | 2.11 (1.30–3.42)   | 0.003             | 1.42 (0.82–2.45)            | 0.21                        |
| >356     | 3.81 (2.36–6.13)   | <0.001            | 2.28 (1.33–3.91)            | 0.003                       |
|          | <0.001†            |                   |                             |                               |

*Nonobese subjects were classified by BMI <30 kg/m²; †trend P values.
level indicated that nonobese subjects with elevated hs-CRP and IL-6 concentrations had an increased likelihood of metabolic syndrome independent of insulin resistance. This suggests that IL-6–induced acute phase responses are likely involved in the pathway of metabolic syndrome and not purely a manifestation of visceral obesity.

Our novel finding with IL-18 further enhances the argument that inflammation and activated immunity are involved in the cluster of metabolic and cardiovascular risk factors. Although its regulation is still poorly understood, IL-18 is now recognized as a central regulator of innate and acquired immune responses. It appears that IL-18 functions as a pleiotropic proinflammatory cytokine, playing an early role in the inflammatory cascade. IL-18 is able to stimulate the production of tumor necrosis factor-α and secondarily IL-6. It may form a link between metabolic syndrome and atherosclerosis because IL-18 is highly expressed in atherosclerotic plaques, and a role in plaque destabilization has been suggested. Prospective studies have shown an association of circulating IL-18 levels with cardiovascular death among patients with coronary artery disease and with coronary events in apparently healthy men.

Until now, IL-18 has not been studied specifically in relation to the metabolic syndrome. However, Esposito et al found that IL-18 levels were raised by acute hyperglycemia in humans through an oxidative mechanism. Plasma IL-18 levels have also been found to be increased in patients with diabetes compared with nondiabetic controls. In relatively small samples of obese premenopausal women, IL-18 levels were found to positively associate with visceral obesity and insulin resistance. In contrast, in the Prospective Epidemiological Study of Myocardial Infarction (PRIME) of apparently healthy European men aged 50 to 69 years, IL-18 levels did not associate with BMI and only weakly with HDL and triglycerides. In our population sample, we found that IL-18 concentrations in both men and women were significantly associated with a range of metabolic risk traits, including BMI, waist circumference, triglycerides, HDL, blood pressure, and fasting insulin levels (Table 2). The strength of our study lies in that a broad cross-section of the general population was represented with males and females across an age range of 27 to 77 years.

Despite associating with individual metabolic traits, IL-18 remained an independent predictor of metabolic syndrome, even after adjustment for obesity and insulin resistance as major determinants of metabolic syndrome. We found a consistent graded relationship between IL-18 concentrations and odds ratios for metabolic syndrome that was independent of age, gender, BMI, and insulin levels. Subjects in the top compared with bottom tertile of IL-18 concentrations had a >2-fold increased odds ratio for metabolic syndrome and a >3-fold odds ratio in nonobese subjects (Table 4). Importantly, the risk relationship between IL-18 and metabolic syndrome was not attenuated after adjustment for hs-CRP and IL-6 in the model.

Several study limitations are acknowledged. Because this is a cross-sectional study, the direction of the association between IL-18, IL-6, and hs-CRP levels and the metabolic syndrome cannot be established. It is possible that metabolic risk traits lead to a heightened inflammatory state rather than being a consequence of chronic inflammation. Nevertheless, our results with IL-18 indicate that the elevated IL-18 levels in patients with the metabolic syndrome are not just a marker of visceral obesity and hyperinsulinemia. It is also possible that a few of our subjects had unrecognized diabetes, but the same immune mechanisms are likely to be involved in the metabolic syndrome and diabetes.

There is now a consistent body of prospective cohort data showing that a variety of inflammatory markers, including hs-CRP and IL-6, predict the development of type 2 diabetes and the incidence of myocardial infarction and cardiovascular death. IL-18 concentrations have already been shown to relate to future CVD, and further prospective studies relating IL-18 concentrations to incident metabolic syndrome, type 2 diabetes, and CVD would be informative. Nevertheless, our current findings with IL-18 support the hypothesis that this pleiotropic proinflammatory cytokine may well be involved in the pathway of metabolic syndrome and form a link between metabolic risk factors, diabetes, and CVD. It may also represent a novel therapeutic target.

Acknowledgments

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