Relation of C-reactive protein to features of the metabolic syndrome in normal glucose tolerant, impaired glucose tolerant, and newly diagnosed type 2 diabetic subjects

F Guerrero-Romero¹, ², M Rodríguez-Morán¹, ²

SUMMARY

Objective: To determine the relationship between CRP levels and the components of MS in normal glucose tolerant (NGT), impaired glucose tolerant (IGT), and Type 2 diabetic subjects.

Material and methods: A based cross-sectional population study was performed. Eligible subjects, men and non-pregnant women, 30 to 64 year of age, were randomly recruited. Subjects with acute or chronic diseases were excluded. Only newly diagnosed type 2 diabetic or hypertensive subjects were included. Disorders related to CRP increase, also were exclusion criteria. In accordance to WHO proposal, components of MS were: High Blood Pressure, Dyslipidemia, Obesity, and Microalbuminuria, and MS was defined, for the NGT, if at least two of the criteria were fulfilled and in addition the subject had insulin resistance. The MS in IGT and DM subjects was defined if at least two of the criteria were fulfilled.

Results: CRP was significantly associated with MS for the NGT (Odds ratio -OR- 3.8, CI 95% 1.6-14.8), IGT (OR 4.9, CI 95% 1.2-15.5), and diabetes (OR 5.6, CI95% 1.9-10.2). For NGT, after adjustment for obesity, CRP was not longer associated with MS. After adjust for obesity and fasting glucose (FG), the relationship between CRP and MS for IGT was lost. Finally, after adjustment for obesity, FG, and microalbuminuria, CRP was not longer associated with MS for diabetic subjects.

Conclusions: This study show a significant relationship between CRP and MS which is maintained only by obesity in the NGT, by obesity and FG in the IGT, and by obesity, FG, and microalbuminuria in the newly diagnosed diabetic subjects.

Key-words: Metabolic Syndrome · C-Reactive Protein · Obesity · Dyslipidemia High Blood Pressure · Microalbuminuria.

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The clustering of risk factors such as obesity, dyslipidemia, high blood pressure (HBP), and hyperglycemia that are related to the development of cardiovascular disease (CVD) and type 2 diabetes mellitus (DM) [1, 2], has been named as metabolic syndrome (MS) [3-5]. The individual components of the MS are well-known independent risk factors for the development of CVD and type 2 DM, thus the presence of a combination of them exert a powerful effect on the risk of the disease [6].

On the other hand, a significant association between inflammation and type 2 DM [7], central fat accumulation [8, 9], insulin resistance [8, 10], dyslipidemia, and atherosclerosis [11], which are components of the MS, has been described, supporting the hypothesis that low-grade chronic inflammation could be related to the development of obesity-associated disorders [8, 9, 12-15].

C-reactive protein (CRP), an acute-phase protein, is the most sensitive marker of inflammation [16]; its increased levels are sufficiently precise to predict CVD [17-19]. In this regard, based on prospective studies has been reported that elevated CRP are associated with atherosclerosis, coronary heart disease [20-22], and diabetes [7].

The NAHINES III data show that CRP are strongly and independently associated to obesity [23]. Adipose tissue release several cytokines that promotes the CRP synthesis [24, 25]. On the other hand, we have previously hypothesized [26] that increased serum glucose, through the sheer stress phenomenon, could produce a systemic endothelial dysfunction and inflammation that might contribute to the elevation of serum markers of inflammation.

These observations have led us to determine the relationship between serum CRP levels and the individual components of MS in normal glucose tolerant (NGT), impaired glucose tolerant (IGT), and newly diagnosed type 2 diabetic Mexican subjects.

Material and methods

With the approval of protocol by the Mexican Social Security Institute Research Committee, and after obtaining subjects informed consent, a based cross-sectional population study was carried out. Eligible subjects, inhabitants of Durango, city in the north of Mexico, were randomly recruited between November 1998 and August 2001. In this regard, a Basic Static Geographic Area, that includes several neighborhoods with similar social and economic characteristics, and a sample of households on it were randomly selected. The chosen households were visited by field workers for inviting to apparently healthy men and non-pregnant women, from 30 to 64 year of age, to participate in the study. A standardized interview and clinical examination was conducted and detailed information on medical history was collected; in addition, all the subjects were laboratorial tested in order to carefully determine the presence of exclusion criteria.

Subjects with acute illnesses as well as people with a history of chronic disease such as high blood pressure, diabetes, or malignancy were excluded. In this regard, only newly diagnosed type 2 diabetic or hypertensive subjects were included. Those disorders related to CRP increase such as, pregnancy, alcohol consumption, cigarette smoking, cardiovascular and coronary heart disease, renal disease, chronic disorders of the joints and connective tissues, and infectious diseases, also were exclusion criteria.

Eligible subjects were allocated into three groups: a) NGT, b) IGT and c) newly diagnosed type 2 DM.

The study was planned to include two control subjects per each subjects with MS. The sample size was estimated according the following criteria: α = 0.05, power = 80%, frequency of elevated CRP levels in the NGT subjects of 10.0% [27], and in the IGT and newly diagnosed diabetes of 19.6% and 35.2% [23]. The required sample size for the subjects with MS were of 138, 112, and 47 in the NGT, IGT, and newly diagnosed diabetes groups, respectively; finally were included 150 NGT, 150 IGT, and 50 diabetic subjects with MS, and its respective controls.

Results of 2-h PG test were categorized according to the American Diabetes Association criteria [28], NGT subjects were required to have both fasting and 2-h post-load serum glucose levels lower than 110 mg/dl and 140 mg/dl, respectively; diagnosis of IGT was based on fasting glucose ≥ 110 mg/dl and < 126 mg/dl, and/or 2-h PG ≥ 140 mg/dl and < 200 mg/dl; and DM was defined by fasting glucose ≥ 126 mg/dl or 2-h post-load glucose ≥ 200 mg/dl. Diagnosis of HBP was established according to the VI Joint National Committee recommendation [29].

In accordance with the WHO proposal [4], the components of the MS are: HBP, defined as systolic/diastolic blood pressure > 160/90 mmHg; Dyslipidemia defined as elevated serum triglycerides (≥ 150 mg/dl) and/or low HDL-cholesterol (< 35 mg/dl in men, < 39 mg/dl in women); Obesity defined as BMI ≥ 30 kg/m² and/or WHR > 0.90 in men, > 0.85 in women; and Microalbuminuria by urinary albumin excretion rate ≥ 30 mg/dl. The MS in NGT subjects was defined if at least two of the criteria listed above were fulfilled and in addition, if the NGT subject was insulin resistant. The MS in the IGT and DM subjects was defined if at least two of the criteria listed above were fulfilled [5]. The homeostasis model insulin analysis resistance (HOMA-IR) index (Fasting glucose mmol/l x Fasting insulin μU/ml/22.5) [30] was used to determine the insulin action. Insulin resistance was defined as the highest quartile of the HOMA-IR index.

Anthropometrics measurements, included height (m), weight (kg), waist (cm), and hip (cm) were measured with the subjects in light clothing and without shoes. Waist cir-
cumference was taken as the minimum circumference at umbilicus level, and hip circumference as the maximum circumference at symphysis of pubis level. Body mass index (BMI) was calculated as the weight divided by height squared, and the waist-to-hip ratio (WHR) as waist circumference divided by hip circumference. All measurements were performed by one of the researchers (MRM). Obesity was defined by both BMI ≥ 30 kg/m² and WHR greater than 0.95 for men and 0.85 for women.

A venous whole blood sample was collected after 8-10 hours of fasting. Serum glucose was measured by the glucose-oxidase method; and the CRP by automated microparticle enzyme immunoassay (IMx, Abbot Laboratories, USA.). The detection limit of CRP was 0.05 mg/dl, with an intra- interassay coefficient of variation of 4.1% and 5.8%, respectively. A serum CRP concentration ≥ 3mg/L defined the elevated CRP levels. The lipid profile was measured by enzymatic methods; the intra- and inter-assay variations were 2% and 3.0%. The first urine sample in the day was collected to determine the urinary albumin excretion, which was determined by nephelometry.

Measurements were performed in an Express 500 clinical chemistry autoanalyzer (Ciba Corning, Diagnostic Corp., Overling, Ohio).

Table I
Characteristics of normal glucose tolerant (NGT), impaired glucose tolerant (IGT), and newly diagnosed type 2 diabetic (DM) subjects, with (+) and without (−) metabolic syndrome (MS).

<table>
<thead>
<tr>
<th></th>
<th>NGT (n = 300)</th>
<th>IGT (n = 300)</th>
<th>DM (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS +</td>
<td>MS −</td>
<td>MS +</td>
<td>MS −</td>
</tr>
<tr>
<td>Age, yr</td>
<td>44.0±10.7</td>
<td>40.8±10.9</td>
<td>45.3±13.0</td>
</tr>
<tr>
<td>Body Mass Index, kg/m²</td>
<td>31.8±2.3</td>
<td>27.4±5.6</td>
<td>33.2±3.3</td>
</tr>
<tr>
<td>Waist-to-Hip ratio</td>
<td>0.94±0.06</td>
<td>0.90±0.07</td>
<td>0.95±0.08</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>124.4±16.8</td>
<td>113.8±22.5</td>
<td>120.2±23.6</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>78.3±10.0</td>
<td>71.9±11.6</td>
<td>75.0±10.7</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>107.9±9.9</td>
<td>90.6±9.3</td>
<td>124.6±21.0</td>
</tr>
<tr>
<td>2-h post-load glucose, mg/dl</td>
<td>110.4±21.4</td>
<td>109.6±17.3</td>
<td>171.5±16.0</td>
</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>7.3±3.8</td>
<td>7.5±3.6</td>
<td>10.2±8.5</td>
</tr>
<tr>
<td>2-h post-load insulin, μU/ml</td>
<td>64.7±37.9</td>
<td>43.2±27.2</td>
<td>89.7±69.7</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>251.8±39.7</td>
<td>215.3±53.4</td>
<td>296.9±55.2</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dl</td>
<td>39.3±14.3</td>
<td>45.8±20.5</td>
<td>36.3±17.5</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dl</td>
<td>175.3±42.6</td>
<td>141.6±48.0</td>
<td>198.3±59.5</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>186.1±81.8</td>
<td>135.8±72.8</td>
<td>281.0±326.8</td>
</tr>
<tr>
<td>Microalbuminuria, mg/dl</td>
<td>26.1±39.4</td>
<td>11.3±49.3</td>
<td>93.1±147.3</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>5.1±1.9</td>
<td>1.8±0.8</td>
<td>3.8±1.2</td>
</tr>
<tr>
<td>C-reactive protein, mg/l</td>
<td>7.0±4.3</td>
<td>0.6±1.3</td>
<td>7.5±6.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD; a p < 0.0001; b p < 0.01; c p < 0.05; d p < 0.001.

Statistical analysis

Differences between the groups were assessed by one-way ANOVA test. Statistical differences between the subgroups with MS and the correspondent control subgroups were established using impaired Student t test.

Pearson’s analysis was performed to examine the correlation between CRP concentration and the components of MS, and Spearman’s rank correlation to establish the correlation between MS and CRP. For the purpose of statistical analysis, all the skewed numerical data were transformed to the Log.

All the components of MS were included as independent variables and the elevated CRP levels (CRP ≥ 3.0 mg/L) as dependent variable in a logistic regression model.

A 95% Confidence interval (CI95%) was considered, and a p value < 0.05 defined the level of statistical significance. The data were analyzed using the statistical package SPSS 8.0 (SPSS Inc., Illinois USA 1998).

Results

One thousand and fifty subjects were included, 450 NGT, 450 IGT, and 150 individuals with DM, of them 350 subjects with diagnosis of MS (150 NGT, 150 IGT, and 50 DM), and 700 control subjects (300 NGT, 300 IGT, and 100
DM). The clinical and metabolic characteristics of the target population are given in Table I. Patients with DM were older than the NGT and IGT subjects. As expected, subjects with MS, showed significantly higher BMI, WHR, total-cholesterol, triglycerides, microalbuminuria, and lower HDL-cholesterol than the correspondent control groups. Blood pressure only showed significant statistical differences between the subjects with and without MS in the NGT and DM groups.

CRP concentration was significantly higher in the subjects with MS (Tab I). The geometric mean (CL$_{95\%}$) of CRP was 8.7 (3.1-10.8) and 1.9 (0.3-2.7) for the subjects with (n = 350) and without (n = 700) MS, respectively.

Fasting glucose showed to be significantly higher in the subjects with MS than the correspondent controls, whereas 2-h post-load glucose showed significant differences only between the subjects with and without MS in the IGT and DM groups. On the other hand, 2-h post-load, but not fasting insulin levels, were significantly elevated in the subjects with and without MS in the NGT and DM groups.

There was a linear increase in the CRP values with an increase in the number of components of the MS. The mean of Ln CRP values (SD) were 0.322 ± 0.05, 0.673 ± 0.15, 0.92 ± 0.1, 1.286 ± 0.2, and 1.435 ± 0.3, for the presence of 0, 1, 2, 3, and 4 components of the MS.

The Pearson’s rank values between CRP concentration and the individual components of MS are given in Table II. Obesity showed a high positive correlation with CRP in the NGT, IGT and DM groups, whereas microalbuminuria was positively correlated with CRP only in the IGT and DM subjects.

Nevertheless that fasting glucose is not a component of the MS by the WHO criteria [4], because it showed significant statistical differences between the subgroups with and without MS we included it in the regression model analysis.

Table III show the Odds ratio calculated by multiple logistic regression analysis. CRP was significantly associated with MS and obesity in all the groups. For the subjects with IGT and DM also CRP was associated with microalbuminuria and fasting glucose.

For the NGT subjects, after adjustment for obesity, CRP was not longer associated with MS. In the IGT group, after adjust for obesity and fasting glucose, the relationship between CRP and MS was not significant. Finally, after adjustment for obesity, fasting glucose, and microalbuminuria, CRP was not longer associated with MS for the newly diagnosed diabetic subjects.

### Discussion

The results of this study show a significant relationship between CRP and MS in the NGT, IGT, and type 2 diabetic subjects, suggesting that chronic, low-grade inflammation could be also part of the MS. In addition, that this study shows is that the independent relationship between MS and CRP is maintained only by obesity in the NGT subjects, by obesity and fasting glucose in the IGT group, and by obesity, fasting glucose, and microalbuminuria in the newly diagnosed type 2 diabetic subjects.

Because both clustering of risk factors named MS and the raised concentration of CRP has been found as predictors for coronary heart disease [1, 4, 31-34], the relationship between MS and CRP has received attention in order to determine whether CRP has a causative role or is only an epiphenomenon in the ischemic heart disease. On the other hand, although has been well-established a strong relationship between CRP and insulin resistance syndrome [35], the nature of its association is not understood at all, and to our knowledge there are not previous reports about the relationship between CRP and MS in NGT, IGT, and newly diagnosed type 2 diabetic subjects.

Taking into account that CRP is an overall marker of inflammation, for determining its relationship with the MS is necessary to control that disorders or conditions likely to trigger an inflammatory response. In this regard, we carefully corroborated the absence of conditions likely to provoke an inflammation, and only included newly diagnosed hypertensive and/or diabetic subjects. Nevertheless, a potential limitation of this study that deserve be mentioned is that we did not measure the exposure to infectious agents such *Helicobacter pylori* and *Chlamydia pneumoniae* that has been related to increased CRP levels [36-38]; however, taking into account the sample size and the enrolling strategy, is unlikely that exposure to these infectious agents could confound the association between CRP levels and MS that was documented.

So, that this study shows is a significant association between CRP levels and MS, relationship that was qualita-
differently different by the NGT, IGT and DM subjects. In all the groups, CRP showed to be related to obesity. In this regard, it is well-documented that the synthesis of CRP in the liver is under the control of Interleukin-6 (IL-6) [39], and TNF-α [40], cytokines that are released or induced by adipose tissue [41], which could be the link between obesity and the elevation of CRP levels. In this way, cytokines arising from adipose tissue could be in part responsible for the low-grade inflammatory status in the subjects with MS.

In addition, both IGT and DM subjects with MS showed a strong and independent relationship between CRP and fasting glucose. Hyperglycemia has been related to changes in the activation of complement [42]; furthermore, previously we have showed that non-controlled type 2 diabetic patients exhibit high CRP levels [43], which although decreased after glycemic control, persisted mild-elevated (unpublished data), supporting the hypothesis that the endothelial shear stress related to the well-known hyperviscosity syndrome linked to the increase of serum glucose, could be the source of the systemic low-grade inflammatory response [43]. Thus, increased CRP concentration in the IGT and diabetic subjects maybe is due to both inflammatory condition of the vascular wall [44, 45], and release of cytokines by adipose tissue.

Observations about the relationship between serum concentrations of CRP and HDL-cholesterol in healthy subjects are inconsistent [35, 46]. In one hand, Yudkin et al. [34] showed a strong and inverse correlation between CRP and HDL-cholesterol concentrations, but on the other hand, Li et al. [46] have reported the absence of such correlation in healthy subjects. Our results showed the presence of an inverse correlation between CRP levels and HDL-cholesterol in the IGT and DM subjects but not in the NGT individuals. This finding might be explained taking into account that in the IGT and DM subjects the inflammatory response could be triggered by several sources, as adipose tissue and endothelium, which determine a significant raise of CRP levels and the decrease of HDL-cholesterol concentration that may reinforce its inverse relationship. In the other hand, since NGT subjects do not show endothelial dysfunction, elevation of CRP seems to be dependent only of obesity [47]; so, in these subjects alterations of both CRP and HDL-cholesterol levels are lower than the observed in subjects with clinical conditions that involve additional sources of inflammation, and thus its correlation tend to be low or absent.

In the same way, the correlation between HOMA-IR index and CRP levels in the NGT subjects, but no in the IGT and DM individuals that we documented might be explained. In this regard, is probable that altered insulin secretion and additional sources of inflammation, that are common among IGT and diabetic subjects, modify in dissimilar

### Table III

Crude and adjusted Odds ratios (confidence interval 95%) calculated by multiple logistic regression analysis in which elevated C-reactive protein level (CRP ≥ 3.0 mg/L) was the dependent variable.

<table>
<thead>
<tr>
<th></th>
<th>NGT n = 450</th>
<th>IGT n = 450</th>
<th>DM n = 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>3.8 (1.6-14.8)</td>
<td>4.9 (1.2-15.5)</td>
<td>5.6 (1.9-10.2)</td>
</tr>
<tr>
<td>Obesity</td>
<td>3.7 (1.3-18.1)</td>
<td>3.2 (1.2-12.2)</td>
<td>3.2 (1.1-9.2)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.1 (0.9-10.3)</td>
<td>1.1 (1.1-10.9)</td>
<td>1.0 (0.9-9.1)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>1.2 (1.0-10.9)</td>
<td>1.1 (1.0-10.8)</td>
<td>1.6 (1.0-9.3)</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>1.0 (0.9-10.7)</td>
<td>1.4 (1.1-10.3)</td>
<td>3.1 (1.3-9.7)</td>
</tr>
<tr>
<td>High quartile of HOMA-IR</td>
<td>1.6 (1.3-12.9)</td>
<td>1.4 (1.1-10.2)</td>
<td>1.2 (1.0-9.1)</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>1.1 (0.8-8.4)</td>
<td>3.0 (1.3-9.6)</td>
<td>4.8 (1.3-8.9)</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age and sex adjusted</td>
<td>3.7 (1.6-9.8)</td>
<td>4.7 (1.2-15.5)</td>
<td>5.5 (1.9-8.7)</td>
</tr>
<tr>
<td>Multiple adjusted 1</td>
<td>1.1 (1.0-9.1)</td>
<td>2.8 (1.1-14.2)</td>
<td>2.9 (1.0-8.6)</td>
</tr>
<tr>
<td>Multiple adjusted 2</td>
<td>1.1 (1.0-8.9)</td>
<td>1.2 (1.0-14.0)</td>
<td>1.8 (1.0-8.2)</td>
</tr>
<tr>
<td>Multiple adjusted 3</td>
<td>1.1 (1.0-8.4)</td>
<td>1.1 (1.0-13.7)</td>
<td>1.1 (0.9-8.1)</td>
</tr>
<tr>
<td>Multiple adjusted 4</td>
<td>1.1 (0.9-8.0)</td>
<td>1.0 (0.9-13.4)</td>
<td>1.0 (0.9-8.0)</td>
</tr>
<tr>
<td>Multiple adjusted 5</td>
<td>1.1 (0.9-7.8)</td>
<td>1.0 (0.9-13.1)</td>
<td>1.0 (0.9-7.9)</td>
</tr>
</tbody>
</table>

NGT = Normal glucose tolerant subjects. IGT = Intolerant glucose subjects. DM = Newly diagnose type 2 diabetic subjects. <sup>a</sup> Odds ratios between MS and CRP levels. Multiple adjusted 1 was adjusted by sex, age, and obesity. Multiple adjusted 2 was adjusted by sex, age, obesity, and fasting glucose. Multiple adjusted 3 was adjusted by sex, age, obesity, fasting glucose, and microalbuminuria. Multiple adjusted 4 was adjusted by sex, age, obesity, fasting glucose, microalbuminuria, and dyslipidemia. Multiple adjusted 5 was adjusted by sex, age, obesity, fasting glucose, microalbuminuria, dyslipidemia, and HOMA-IR index.

C-Reactive protein and metabolic syndrome

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magnitudes both HOMA-IR index values and CRP levels, which could alter its relationship. On the other hand, concentrations of CRP and HOMA-IR index values in the NGT subjects are both obesity-dependent and there are not influenced by other sources of inflammation or alteration on insulin secretion; so, seems to be that the strong correlation between HOMA-IR and CRP levels in the NGT subjects is fundamentally obesity-related. In this concern, reports are consistent showing a significant correlation between CRP levels and insulin resistance in apparently healthy subjects [8, 10, 48]. In addition, in this study, after controlling by obesity the association between CRP and HOMA-IR in the NGT subjects disappear, finding that support our putative explanation.

Finally, our data show that microalbuminuria is independently related to increased CRP levels in the newly diagnosed type 2 diabetic individuals, but not in the IGT and NGT subjects. Microalbuminuria reflect a generalized vascular damage [49] and is a well-established cardiovascular risk factor [50]. According to our hypothesis, a chronic exposure to mild or severe hyperglycemia increase the shear stress producing a systemic endothelial dysfunction and inflammation, which is clinically evident by microalbuminuria and raised inflammatory cytokines that promotes hepatic production of CRP. In this way, both microalbuminuria and increased CRP levels would be related to mechanisms of endothelial inflammation induced by sustained hyperglycemia. Because type 2 diabetic subjects have had a greater exposure to the sustained hyperglycemia than IGT or NGT individuals, they show a more advanced stage of endothelial dysfunction and inflammation ant thus high CRP and microalbuminuria.

However, because on cross-sectional studies is not possible to attribute causality nor establish pathophysiological mechanisms, the strong relationship between MS and some of its components with elevated CRP levels as well as our putative explanation, based on the shear stress, for the increase of CRP levels, will require further testing.

**References**


