C-reactive protein is independently associated with glucose but not with insulin resistance in healthy men

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Additional information for this article can be found in an online appendix at http://care.diabetesjournals.org.

Anne G. Niehoff, MD1,2
Timon W. van Haeften, MD, PhD3
N. Charlotte Onland-Moret, PhD1,2
Clara C. Elbers1,2
Cisca Wijmenga, PhD2,4
Yvonne T. van der Schouw, PhD1

1Julius Center for Health Sciences and Primary Care
2Complex Genetics Section, DBG-Department of Medical Genetics
3Department of Internal Medicine
University Medical Center Utrecht, the Netherlands
4Present address: Department of Genetics, University Medical Center Groningen, the Netherlands

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Correspondence:
Yvonne T. van der Schouw, PhD
E-mail: y.t.vanderschouw@umcutrecht.nl
Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Room 6.131 Stratenum, PO Box 85500, 3508 GA Utrecht, the Netherlands
Recent studies have shown that low-grade inflammation is linked to both insulin resistance (1; 2) and the metabolic syndrome (MetS) (3; 4) and may even predict the development of type 2 diabetes (T2D) (5-8).

However adipose tissue might play an important role in these relationships. There is still controversy about whether low-grade inflammation is an intermediate factor between obesity and insulin resistance, or whether it has an independent effect on the development of T2D through a mechanism separate from obesity.

The aim of this study was to explore whether low-grade inflammation (measured by plasma C-reactive protein (CRP) levels) was related to insulin resistance and to parameters of MetS, independently of obesity.

**Research Design and Methods**

We conducted a cross-sectional, single-centre study, on 400 men aged between 40 and 80 years. The subjects, methods of recruitment, study procedures and anthropometrical and laboratory measurements have been described elsewhere (9). Twenty-one participants had prevalent diabetes mellitus and were excluded from the study, 13 participants were excluded because their level of CRP was above 10 mg/l and two because blood samples had been taken in a non-fasting state. Our final study population comprised 364 participants.

Various measures of obesity were obtained: weight, BMI, waist circumference (WC), waist-to-hip ratio (WHR), visceral and subcutaneous fat using ultrasound measurement (10) and total body fat mass using dual-energy x-ray absorptiometry.

Information on prevalent diseases, medication use and life-style factors was available. Peripheral blood pressure was measured. Physical activity was assessed using the Voorrips score (11).

Hs-CRP, HDL cholesterol, triglycerides, glucose and insulin where measured in fasting blood samples. Details about the precision of the assays have been reported (9; 12; 13). Insulin sensitivity was determined with the homeostasis model assessment of insulin resistance (HOMA-IR): [(fasting insulin (mU/l) * fasting glucose (mmol/l))/22.5. The presence of MetS was defined according to the National Cholesterol Education Program criteria (14).

**Statistical analysis**

Subjects were divided into quartiles according to their CRP level. Linear regression analysis was used to estimate the relation of CRP with insulin resistance and the individual components of MetS. The p-value for linear trend was calculated with the median of the CRP level in each quartile. Multivariate analyses were adjusted for age, smoking and physical activity. To explore the relationship between CRP and insulin resistance, and between CRP and MetS independently of markers of obesity, the models were also adjusted for various different body composition parameters. Statistical analyses were performed using SPSS, version 12.0.1 for Windows.

**Results**

In general, men with higher CRP levels were older, scored higher on markers of obesity, were less physically active, and had higher glucose levels and insulin resistance. They also fulfilled the criteria for MetS more often (Online Appendix.
Table 1 [available at http://care.diabetesjournals.org]).

After adjusting for confounders (age, smoking and physical activity), insulin resistance was significantly higher in the 4th quartile of CRP compared to the 1st, but after adjusting for the various parameters of obesity, the difference in HOMA-IR between the quartiles of CRP was no longer evident (Table 1). However, adjustment for subcutaneous fat attenuated the relationship between CRP and HOMA-IR to a lesser extent than the other parameters of obesity.

Of the individual components of MetS, WC, glucose and HDL-cholesterol showed a statistically significant association with CRP (data not shown). When we further adjusted the relationship between CRP and the individual components of MetS for measures of obesity, only glucose remained statistically significantly higher in the 4th quartile of CRP compared to the 1st quartile (Table 1).

Conclusions
CRP was not related to insulin resistance after adjustment for parameters of body composition, whereas CRP was independently associated with glucose, but not with the other components of MetS.

Recently Kahn et al (15) showed that the relationship between CRP and the number of components of MetS markedly attenuated and became non-significant after adjusting for BMI in drug-naive T2D patients. Because all the patients in their study satisfied the glucose criteria of MetS by definition, this criterion did not contribute to the correlation between CRP and components of MetS. Our study therefore broadens what is known about this topic, since it shows that CRP is associated with plasma glucose, independently of obesity. Furthermore, in healthy elderly men we confirmed Kahn et al’s finding that CRP is not independently related to other components of MetS.

Conflicting results have been found regarding the correlation between CRP and insulin resistance (2; 16-20). However most of these studies did not have the prime aim of exploring the underlying pathways. Some of these studies did not therefore adjust for parameters of obesity at all, while others did not investigate the effect of a separate adjustment for obesity.

A limitation of our study is its cross-sectional design and therefore we can only suggest, but not prove, the causality of the observed relationships. We used HOMA-IR and ultrasound instead of hyperinsulinemic euglycemic clamp and CT scan, respectively, for assessing insulin sensitivity and visceral and subcutaneous fat. However, these measurements have proven to be strongly correlated with the gold standards (correlation coefficients of -0.82 and 0.81, respectively (10; 21)).

Our data point to an obesity-independent mechanism which relates CRP and glucose. One explanation might be the pro-inflammatory effects of glucose: in itself glucose is pro-inflammatory (22), and it can increase IL-6, TNF-α and IL-18 release in healthy subjects and persons with impaired glucose tolerance (23). Furthermore, prolonged hyperglycaemia and the accompanying production of excess amounts of advanced glycation end products (AGEs) can activate NF-κB (24). This will lead to an inflammatory reaction, independently of adipose tissue.

In conclusion, our findings strongly point to low-grade inflammation being an intermediate factor in the relationship between adipose tissue and
insulin resistance. The independent association of glucose with CRP suggests an additional mechanism by which glucose and CRP are related, independently of obesity.

Acknowledgments
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References
15. Kahn SE, Zinman B, Haffner SM, O'Neill MC, Kravitz BG, Yu D, Freed MI, Herman WH, Holman RR, Jones NP, Lachin JM, Viberi GC: Obesity is a major determinant of
the association of C-reactive protein levels and the metabolic syndrome in type 2 diabetes. *Diabetes* 55:2357-2364, 2006


Table 1. Relationship between C-Reactive Protein (CRP) (in quartiles) and insulin resistance as assessed by HOMA-IR, and between C-Reactive Protein (in quartiles) and glucose, with the first quartiles as reference.

<table>
<thead>
<tr>
<th></th>
<th>Insulin resistance; β-coefficient (95% CI) per quartile of CRP</th>
<th>Glucose; β-coefficient (95% CI) per quartile of CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Adjusted confounders</td>
<td>for ref</td>
<td>0.32 (-0.13;0.77)</td>
</tr>
<tr>
<td>Adjusted weight†</td>
<td>for ref</td>
<td>0.09 (-0.33;0.51)</td>
</tr>
<tr>
<td>Adjusted WC†</td>
<td>for ref</td>
<td>0.02 (-0.38;0.42)</td>
</tr>
<tr>
<td>Adjusted WHR†</td>
<td>for ref</td>
<td>0.07 (-0.36;0.49)</td>
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<tr>
<td>Adjusted BMI†</td>
<td>for ref</td>
<td>0.13 (-0.26;0.53)</td>
</tr>
<tr>
<td>Adjusted visceral fat†</td>
<td>for ref</td>
<td>0.18 (-0.23;0.58)</td>
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<tr>
<td>Adjusted subcutaneous fat†</td>
<td>for ref</td>
<td>0.07 (-0.37;0.51)</td>
</tr>
<tr>
<td>Adjusted percentage mass†</td>
<td>for ref</td>
<td>0.02 (-0.38;0.42)</td>
</tr>
</tbody>
</table>

*CRP quartile ranges are: (1) 0-0.6 mg/l, (2) 0.6-1.2 mg/l, (3) 1.2-2.6 mg/l, (4) 2.6-10 mg/l.
† All analyses are adjusted for confounders; age, physical activity and smoking. ‡ p-value for trend.