Autoantibodies against oxidized low-density lipoprotein and C-reactive protein are associated with diabetes and myocardial infarction in women

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ABSTRACT

Women with diabetes mellitus are at high risk of myocardial infarction (MI), and it is well recognized that smoking, hypertension, hyperlipidaemia and the diabetic state itself do not fully explain this increased risk. During the last decade, growing evidence has accumulated that the immune system, with oxidized low-density lipoprotein (LDL) as a key antigen, plays an important role in the development of atherosclerosis. The aim of the present study was to explore the association between the immune response, as measured by antibody titres to malondialdehyde-treated LDL (MDA-LDL) and levels of C-reactive protein (CRP; a marker of inflammation), and diabetes mellitus and MI in women. Women (35–64 years) with diabetes (n = 18) and non-diabetic women (n = 46) who had been treated in hospital for MI were compared with diabetic women without MI (n = 35) and healthy controls (n = 70). Blood samples were collected after an overnight fast. CRP was determined with a highly sensitive immunoenzymometric assay. IgM and IgG antibodies against MDA-LDL were analysed with a solid-phase ELISA technique. Women with diabetes but without previous MI were more similar to women with previous MI (both with and without diabetes) than to the healthy controls. Compared with healthy women, the women with diabetes and/or MI had higher IgG (P < 0.05) and lower IgM (P < 0.006) antibody titres against oxidized LDL and higher CRP levels (P < 0.001), associations that were independent of other cardiovascular risk factors. These findings might indicate a differentiated immune response against modified LDL, more pronounced inflammation and a more aggressive atherosclerotic process in women with diabetes.

INTRODUCTION

A considerable proportion of patients with diabetes (values of 50–70% are typical) are affected by cardiovascular disease (CVD) during their life span. Previous studies have shown a severalfold increased risk of primary and recurrent myocardial infarction (MI) and of cardiovascular mortality in diabetic compared with non-diabetic women, women with diabetes and MI; DM-not-MI women, diabetic controls; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; ICS, internal control sample; ISS, internal standard sample; LDL, low-density lipoprotein; MDA-LDL, malondialdehyde-treated LDL; MI, myocardial infarction; MI-not-DM women, non-diabetic women with MI; ox-LDL, oxidized LDL; SBP, systolic blood pressure; WHR, waist/hip ratio.

Key words: autoantibodies, diabetes mellitus, oxidized LDL, women.

Abbreviations: ACE, angiotensin-converting enzyme; BMI, body mass index; CHD, coronary heart disease; CI, confidence intervals; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; DM + MI women, women with diabetes and MI; DM-not-MI women, diabetic controls; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; ICS, internal control sample; ISS, internal standard sample; LDL, low-density lipoprotein; MDA-LDL, malondialdehyde-treated LDL; MI, myocardial infarction; MI-not-DM women, non-diabetic women with MI; ox-LDL, oxidized LDL; SBP, systolic blood pressure; WHR, waist/hip ratio.

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diabetic subjects [1–8]. Furthermore, the difference in morbidity and mortality due to coronary heart disease (CHD) between diabetic and non-diabetic individuals seems to be more prominent in women than in men [1–2,4,7], but findings have not been entirely consistent [8].

Most prior studies of risk factors for CVD in diabetes mellitus have reported increased levels of serum total cholesterol, low-density lipoprotein (LDL)-cholesterol, triacylglycerols and blood pressure, and lower levels of high-density lipoprotein (HDL)-cholesterol, in diabetic compared with non-diabetic subjects. However, these traditional risk factors, or the diabetic state itself, cannot fully explain the excess risk of MI and its complications in women with diabetes [1].

The immune system has emerged over the last few years as a key factor in the development of atherosclerosis. Modified lipoproteins, and especially oxidized LDL (ox-LDL), have been the focus of much research. Ox-LDL may be a key antigen in the atherogenic process, by stimulating monocytes to infiltrate into the vessel intima; these then differentiate into macrophages, which produce cytokines, oxygen radicals and heat-shock proteins, and form foam cells [9].

Several studies have supported the hypothesis that ox-LDL is involved in the development of atherosclerosis in humans. Antibodies against ox-LDL have been demonstrated in human and rabbit atherosclerotic lesions [10]. In young male MI survivors, the lag phase of LDL oxidation was inversely associated with coronary atherosclerosis [11]. In addition, increased levels of antibodies against ox-LDL were predictive of MI [12] and the progression of carotid atherosclerosis [13] in men. However, in other studies, no association with cardiovascular events [14], carotid intima-media thickness [14,15] or the extent of coronary atherosclerosis [16] was found.

The demonstration of antibodies against ox-LDL indicates the occurrence of lipid peroxidation in vivo. Lipid peroxidation may be increased in diabetes, as hyperglycaemia influences the production of free radicals by auto-oxidation of glucose and the formation of advanced glycation end-products and superoxide radicals [17]. Previous studies have reported elevated levels of antibodies against modified LDL [18–21] in diabetic compared with non-diabetic subjects; however, divergent results have also been reported [14,22]. Furthermore, no previous studies have examined diabetic women specifically.

C-reactive protein (CRP) is considered to be a sensitive marker for systemic inflammation, and is synthesized by the liver in response to cellular cytokines [23]. It is associated with several CVD risk factors [24,25], and is also an independent risk factor for CVD in both men and women [26,27].

The aim of the present study was to explore the association between the immune response, as measured by antibody titres to malondialdehyde-treated LDL (MDA-LDL), CRP, diabetes mellitus and MI in women.

**METHODS**

**Study subjects**

The protocol was approved by the Ethics Committee of Göteborg University, and informed consent was obtained from all participants.

**Women with and without diabetes, having suffered an MI**

Göteborg, the second largest city in Sweden, is situated on the west coast of the country, and has a population of about 450 000. The Göteborg Myocardial Infarction Register, which was started in 1970, monitors all events of MI and deaths from CHD in individuals below 65 years of age in the city [29]. All surviving women below the age of 65 years who had been treated for an MI in the two Göteborg hospitals between 1994 and 1996 were invited to participate in the study. MI was considered to have occurred when at least two of the three criteria of chest pain, enzyme leakage and typical ECG changes had been fulfilled, in accordance with criteria of the Swedish Society of Cardiology.

The prevalence of diabetes was not known before examination. As there turned out to be few surviving women with diabetes and MI (DM + MI women) that could be included, we also invited DM + MI women from two neighbouring hospitals to participate. In all, 29 out of 36 (81%) invited DM + MI women answered the questionnaire, and 22 participated initially in physical examination and blood sampling (see Measurements section below). In addition, 64 out of 82 (78%) non-diabetic women with MI (MI-not-DM women) answered the questionnaire, and 52 participated in physical examination and blood sampling.

The analysis of antibodies to MDA-LDL and CRP levels was performed on frozen samples 3 years after initial screening. Unfortunately, some samples were missing, which is why data from only 18 DM + MI and 46 MI-not-DM women could be included. However, a comparison of anthropometric and lipid variables between these women and those for whom samples were missing showed similar results (results not shown). The time interval between MI and examination varied between 2 and 40 months.

**Diabetic women without MI and controls**

In 1985, 1990 and 1995, random samples of the Göteborg population were examined within the framework of the WHO-MONICA project, a multinational study initiated to register risk factors and trends in the incidence of CVD in the general population [28]. In each of these screenings, 557 (75%), 640 (73%) and 692 (66%) respectively of the originally invited women (35–64 years of age) were examined. A random subsample of 70 women from the
1995 MONICA screening constituted the healthy controls in the present study. Women with diabetes but without previous MI from the three MONICA screenings (\(n = 20\)) and women with diabetes from the hospital outpatient clinic (\(n = 15\)) constituted the diabetic controls (DM-not-MI women) (\(n = 35\)). There were no major differences for any variable measured between these two groups of diabetic women.

Diabetes was defined as an affirmative response to the question ‘Did a doctor tell you that you have diabetes?’ and/or anti-diabetic treatment with diet, oral agents and/or insulin. None of the control women had a history of previous MI, and all had a normal resting ECG. With a few exceptions, all women in the study were examined within a period of 15 months.

**Measurements**

The screening methods for the MONICA study were also used for women in the present study with diabetes and/or MI, and are described in detail elsewhere [30]. Briefly, an invitation letter with information about the study and a postal questionnaire on present and past health status, smoking habits and medication was sent to the participants. After an overnight fast, a physical examination was performed, blood pressure was measured and blood samples were collected, whereafter the participants were interviewed by a physician.

Smoking habits were graded as: (1) never smoked, (2) ex-smoker of more than 1 month’s duration, and (3) current smoker. Height, weight and circumferences of the waist and hip were measured with the subject standing, in light clothing, to allow calculation of body mass index (BMI) and waist/hip ratio (WHR). Blood pressure was measured in the sitting position on two occasions, after at least a 5 min rest, to the nearest 2 mmHg. Venous blood samples were drawn for analysis on the same day of glycated haemoglobin (HbA\(_c\)), total cholesterol, HDL-cholesterol and triacylglycerol. Further blood samples were collected and serum was kept frozen at \(-70^\circ\text{C}\). With respect to the lipid analyses, the laboratory was standardized against the MONICA laboratory in Prague. HbA\(_c\) was analysed with an HPLC method (normal range 3.6–5.3 %).

**Antibody titres against modified lipoproteins**

**Determination of antibody titres against modified lipoproteins**

Antibody titres were determined with a solid-phase ELISA, as described previously [15]. Antibody titre was defined as:

\[
\text{Titre} = \frac{\text{absorbance (patient serum – post-coat)}}{\text{internal antibody titre standard serum – post-coat}}.
\]

For IgG, the post-coated wells gave no absorbance; therefore this correction was made only for IgM.

**Internal antibody titre standard used**

On each plate, two different internal standard serum samples were used repeatedly. The absorbances of these two samples, named the internal control sample (ICS) and the internal standard sample (ISS), were used to calculate the ICS/ISS ratio, which was used as the internal antibody titre standard. When using previously described predefined criteria for re-analysing titres, the variability has been shown to be satisfactory [15]. S.D.s for the mean value of the ICS/ISS ratio (i.e. internal antibody titre standard used) from all plates were 0.03 for IgG and 0.06 for IgM titres against MDA-LDL when the predefined criteria were used.

**Determination of CRP concentration**

For the quantitative determination of CRP in serum, a commercially available, highly sensitive immuno-enzymometric assay (Oy Medix Biochemica AB, Kauniainen, Finland), modified at the Wallenberg laboratory, was used. Monoclonal antibodies specific for human CRP were immobilized on microwell plates. Labelled monoclonal antibodies were prepared by conjugating to horseradish peroxidase. The minimum CRP concentration detectable by the method was 0.2 mg/l.

**Statistical methods**

Analyses were performed using SAS statistical software, version 6.12. Age-adjusted means for anthropometric data, blood pressure and lipid levels were determined by analysis of covariance, with age as the dependent covariate. Age-adjusted means for antibody titres against MDA-LDL and CRP are presented in the text as mean ± 95 % confidence intervals (CI). As CRP levels were not normally distributed, the non-parametric Spearman’s correlation test was used to test correlations between CRP and anti-LDL antibodies, and between CRP, anti-LDL antibodies and CVD risk factors. Multiple regression was used to test the association between diabetes and MI on the one hand and antibody titres and CRP on the other with respect to background variables such as BMI, WHR, systolic blood pressure (SBP), triacylglycerol, HDL-cholesterol, medication and menopause.

**RESULTS**

**Characteristics of study subjects**

Age, smoking habits, menopausal state, duration of diabetes, HbA\(_c\) levels, anthropometric data, SBP, and serum total cholesterol, triacylglycerol and HDL-chol-
Table 1  Characteristics of study subjects
Values are means (95% CI).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DM + MI (n = 1B)</th>
<th>MI-not-DM (n = 46)</th>
<th>DM-not-MI (n = 35)</th>
<th>Controls (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.4 (54.2, 60.6)</td>
<td>57.3 (55.0, 59.5)</td>
<td>56.5 (54.4, 58.7)</td>
<td>50.6 (48.8, 52.5)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>44.5</td>
<td>13.4</td>
<td>20.0</td>
<td>37.1</td>
</tr>
<tr>
<td>Ex-smokers (%)</td>
<td>38.9</td>
<td>60.9</td>
<td>22.9</td>
<td>22.9</td>
</tr>
<tr>
<td>Postmenopausal (%)</td>
<td>100.0</td>
<td>80.0</td>
<td>82.9</td>
<td>47.7</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>17.2 (9.4, 25.0)</td>
<td>–</td>
<td>16.2 (14.1, 20.8)</td>
<td>–</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.06 (7.08, 9.04)</td>
<td>–</td>
<td>7.22 (6.58, 7.86)</td>
<td>–</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>27.8 (25.6, 30.1)</td>
<td>26.1 (24.7, 27.5)</td>
<td>26.9 (25.1, 28.6)</td>
<td>25.3 (24.2, 26.5)</td>
</tr>
<tr>
<td>WHR*</td>
<td>0.89 (0.86, 0.92)</td>
<td>0.85 (0.83, 0.86)</td>
<td>0.84 (0.82, 0.86)</td>
<td>0.80 (0.79, 0.82)</td>
</tr>
<tr>
<td>SBP (mmHg)*</td>
<td>146 (137, 154)</td>
<td>133 (128, 138)</td>
<td>139 (134, 144)</td>
<td>129 (125, 133)</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)*</td>
<td>5.93 (5.32, 6.54)</td>
<td>5.87 (5.50, 6.24)</td>
<td>6.39 (5.97, 6.81)</td>
<td>6.29 (5.99, 6.60)</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol/l)*</td>
<td>1.11 (0.89, 1.34)</td>
<td>1.27 (1.14, 1.41)</td>
<td>1.51 (1.35, 1.66)</td>
<td>1.67 (1.56, 1.78)</td>
</tr>
<tr>
<td>Serum triacylglycerols (mmol/l)*</td>
<td>3.08 (2.53, 3.62)</td>
<td>1.80 (1.47, 2.13)</td>
<td>1.69 (1.32, 2.07)</td>
<td>1.51 (1.24, 1.79)</td>
</tr>
</tbody>
</table>

* Age-adjusted means.

Figure 1  Titres of IgM and IgG antibodies against MDA-LDL in the DM + MI, MI-not-DM and DM-not-MI groups, and in healthy control women

esterol levels in the participating women are shown in Table 1. Mean age was lower among the control women, since in the MONICA study similar numbers of women in each 10-year age group (i.e. 35–44, 45–54 and 55–64 years) were included. Among the patients, one DM + MI, three MI-not-DM and one DM-not-MI women were under 45 years of age. The lower mean age in the control group was reflected in the difference in menopausal state between the groups. Age at menopause was similar among the four groups of women (results not shown).

More women who had sustained an MI were smokers and ex-smokers compared with women without MI. Diabetes duration and metabolic control were similar in the two groups of women with diabetes.

The mean values of BMI, WHR, SBP and lipids were adjusted for age. DM + MI women had significantly higher values for BMI ($P < 0.001$), WHR ($P < 0.001$), SBP ($P < 0.001$) and triacylglycerol ($P < 0.001$), and lower HDL levels ($P < 0.001$), compared with controls. Furthermore, WHR ($P < 0.05$), SBP ($P < 0.01$) and triacylglycerol ($P < 0.001$) were significantly higher in DM + MI than in MI-not-DM women, and WHR was significantly higher ($P < 0.01$) and HDL significantly lower ($P < 0.01$) than in DM-not-MI women.

MI-not-DM women had significantly higher WHR ($P = 0.001$) and lower HDL levels ($P < 0.001$) than controls, and significantly lower HDL levels ($P < 0.05$) than DM-not-MI women.

DM-not-MI women had significantly higher WHR ($P < 0.01$) and SBP ($P = 0.01$) values than controls, and HDL-cholesterol tended to be lower, but not significantly so. Diastolic blood pressure (DBP) was similar in the four groups.

**Antibody titres to MDA-LDL and CRP levels**

IgM and IgG titres against MDA-LDL in the four groups of women are shown in Figure 1. The MI + DM group tended to have lower IgM titres than the controls (means ± 95% CI: 1.41 ± 0.17 and 1.61 ± 0.10 respectively; $P = 0.053$). MI-not-DM (1.42 ± 0.11; $P < 0.05$) and DM-not-MI (1.45 ± 0.12; $P < 0.05$) women had significantly lower IgM titres to MDA-LDL compared with controls.

No significant differences in IgG titres were seen between MI + DM women and controls (0.98 ± 0.09 and 0.95 ± 0.05 respectively). However, IgG titres were significantly higher in MI-not-DM women (1.07 ± 0.05) than in controls ($P = 0.001$). The DM-not-MI group (1.02 ± 0.06) tended to have a higher IgG titre than controls ($P = 0.07$).

CRP levels were significantly higher in the MI + DM women (7.81 ± 2.4 mg/l) than in the MI-not-DM group (3.58 ± 1.53 mg/l; $P < 0.01$) and in controls.
In the merged group of patients (cases), CRP and IgG titres were significantly higher compared with controls (Figures 1 and 2; Table 2). On the other hand, IgM titres were significantly lower than in controls (Figures 1 and 2; Table 2). Adjustment for menopausal state in the analyses of IgG and IgM titres did not change the results, and nor did logarithmic transformation of CRP levels (results not shown).

Influence of medication
Among women with previous MI (n = 64), 34% were treated with angiotensin-converting enzyme (ACE) inhibitors and 53% with lipid-lowering drugs. In the merged group of patients and controls (n = 169), 97% of ACE inhibitors (n = 30) and 88% of lipid-lowering drugs (n = 36) were prescribed to women with previous MI and/or diabetes. Women on ACE inhibitors or lipid-lowering drugs had lower IgM and higher IgG titres compared with the women without medication. However, regression analyses with IgM and IgG as dependent variables revealed that this was explained by diabetes and/or MI, and not by medication.

Correlation analyses and multiple regression
CRP was negatively correlated with IgM titre in control women (r = −0.24, P < 0.05), but not in cases, and showed no association with IgG titre. There was no association between IgG and IgM titres.

The correlation coefficients for the associations between IgG titre, IgM titre and CRP levels with age, BMI, WHR, blood pressure and lipid levels in cases and controls are shown in Table 3. When cases and controls

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**Table 2** IgM and IgG titres of antibodies against MDA-LDL and CRP levels in cases and healthy controls
Cases comprise women with diabetes and/or MI. Values are age-adjusted means (95% CI).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (n = 99)</th>
<th>Controls (n = 70)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM titre</td>
<td>1.43 (1.35, 1.50)</td>
<td>1.61 (1.51, 1.70)</td>
<td>0.005</td>
</tr>
<tr>
<td>IgG titre</td>
<td>1.03 (1.00, 1.07)</td>
<td>0.95 (0.90, 0.99)</td>
<td>0.005</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>4.75 (3.55, 5.94)</td>
<td>2.16 (1.29, 3.03)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

(1.90±1.31 mg/l; P < 0.001). Compared with controls, CRP was significantly higher in MI-not-DM women (P < 0.05) and DM-not-MI women (5.30±1.74 mg/l; P < 0.001) (Figure 2). There were no significant differences between the DM-not-MI women and the groups with MI (MI-not-DM and MI + DM) in either antibody or CRP levels.

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**Table 3** Spearman correlation coefficients for the associations of IgM titres, IgG titres and CRP levels with age, BMI, WHR, SBP, DBP and lipid levels in women with diabetes and/or MI (cases; n = 99) and controls (n = 70)
Significance: *P < 0.05, **P < 0.01, ***P < 0.001.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IgG</th>
<th>IgM</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>−0.20*</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.02</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>0.05</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>0.00</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>0.09</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>0.03</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Serum triglycerols</td>
<td>−0.14</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>0.09</td>
<td>−0.05</td>
<td></td>
</tr>
</tbody>
</table>
were analysed together (results not shown), IgG titre was correlated with DBP \( (r = 0.16, P < 0.05) \), and IgM titre was negatively associated with BMI \( (r = -0.27, P < 0.001) \), WHR \( (r = -0.21, P < 0.01) \), SBP \( (r = -0.24, P < 0.01) \) and DBP \( (r = -0.19, P < 0.05) \). Neither IgG nor IgM titres were associated with age, lipid levels or duration of diabetes.

Furthermore, when cases and controls were analysed together, CRP level was associated with age \( (r = 0.18, P < 0.05) \), BMI \( (r = 0.40, P < 0.001) \), WHR \( (r = 0.44, P < 0.001) \), SBP \( (r = 0.26, P < 0.001) \), serum triacylglycerols \( (r = 0.34, P < 0.001) \) and HDL-cholesterol \( (r = -0.31, P < 0.001) \). Moreover, CRP was positively correlated with HbA1c \( (r = 0.25, P = 0.038) \). Neither CRP nor antibodies to ox-LDL were associated with smoking in the present study.

In multiple regression analysis (Table 4), the association of cases (i.e. women with diabetes, MI or both) with high IgG titres, low IgM titres and high CRP levels was independent of other CVD risk factors. However, BMI (for IgM and CRP) and triacylglycerols (for IgG and CRP) accounted for some of the differences between cases and controls.

**DISCUSSION**

The present study shows that women with an increased risk of atherosclerosis (i.e. with diabetes, previous MI or both) had high CRP levels, and high IgG titres and low IgM titres to MDA-LDL, compared with a healthy control group. Furthermore, CRP was positively associated with BMI, WHR, blood pressure and triacylglycerols, and negatively associated with HDL-cholesterol and IgM titre to MDA-LDL.

We have no reason to believe that increased mortality among diabetic women with MI, resulting in low numbers of participants, would have influenced the differences between the groups. Instead, the increased mortality probably leads to underestimation rather than exaggeration of the differences.

As the incidence of CVD increase with age, the studied groups could have been larger if the upper age limit had been higher. However, the rationale for choosing 64 years of age as the limit was the advantage of using the MI register and the MONICA population when recruiting subjects for the study. The MI register made it possible for us to identify all women who had been hospitalized for an MI during the period 1994–1996. The MONICA population is preferable as a control group, as it is a random sample of the general population. However, neither the MI register nor the MONICA population comprises subjects older than 64 years.

Another possible objection to the study could be that both women with Type I diabetes and those with Type II diabetes were included. However, to separate the analyses would have resulted in smaller groups. Furthermore, increased levels of antibodies against ox-LDL have been reported previously in subjects with both types of diabetes [18–21]. Therefore we found it reasonable to include women with either Type I or Type II diabetes.

In contrast with the patients, who were recruited consecutively, the healthy control subjects were selected randomly from a general population sample having similar numbers of participants in each 10-year age group between 35 and 64 years of age. This resulted in more women aged 35–45 years in the control group than in the other groups, thereby lowering the mean age in that group. We refrained from matching the patients and controls for age or other variables, as information might get lost for each procedure of matching, but we adjusted for age and other relevant factors in the analyses when appropriate.

The most consistent finding in the present study was that women with diabetes and/or previous MI had lower IgM titres to MDA-LDL and higher CRP levels compared with the healthy control subjects. It is well known

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**Table 4 Multiple regression analyses of IgM and IgG titres and log CRP levels on CVD risk factors**

Risk factors are case, BMI, WHR, SBP, triacylglycerols, HDL-cholesterol and menopause. Cases are defined as women with diabetes and/or MI. Only variables significant at the 0.15 level are left in the model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Risk factor</th>
<th>Parameter</th>
<th>Standard error</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>Case</td>
<td>-0.20</td>
<td>0.059</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>-0.016</td>
<td>0.006</td>
<td>0.014</td>
</tr>
<tr>
<td>IgG</td>
<td>Case</td>
<td>0.066</td>
<td>0.012</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>Triacylglycerols (mmol/l)</td>
<td>-0.028</td>
<td>0.012</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>Menopause</td>
<td>0.050</td>
<td>0.034</td>
<td>0.14</td>
</tr>
<tr>
<td>log([CRP (mg/l)])</td>
<td>Case</td>
<td>0.610</td>
<td>0.214</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>0.089</td>
<td>0.025</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>WHR</td>
<td>2.73</td>
<td>1.880</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Triacylglycerols (mmol/l)</td>
<td>0.264</td>
<td>0.088</td>
<td>0.003</td>
</tr>
</tbody>
</table>
that patients with diabetes are characterized by increased occurrence of atherosclerosis. Inflammation has been postulated to play an important role in the development of atherosclerosis [31]. Several lines of evidence support the view that modified LDL may be a key antigen in atherogenesis. However, so far it has not been established whether the immune response to modified LDL is pro-atherogenic or anti-atherogenic in vivo [9,32]. The general hypothesis has been that high antibody titres to modified LDL are pro-atherogenic [12,13]. However, reduced levels of antibodies to ox-LDL were reported in patients with ischaemic stroke [33], in patients with acute MI [34], and during postprandial lipaemia in atherosclerotic patients [35]. Also in support of the concept that the immune response might be anti-atherogenic rather than pro-atherogenic are reports indicating that immunization of experimental animals with modified LDL leads to dramatically enhanced IgG levels and inhibits the progression of atherosclerosis [36–38]. B-cells can produce IgM without the help of T-cells. Shaw et al. [39] recently demonstrated that naturally occurring IgM antibodies, structurally and functionally identical to antibodies of B-cell origin protecting against pneumococcal infections, block the macrophage uptake of ox-LDL in apolipoprotein E-deficient mice. A high titre of IgM to ox-LDL among the healthy women in the present study might thus reflect T-cell-independent B-cell activation with a protective function. The lower IgM titres among the diseased women in the present study are in accordance with a previous study in which prior MI was associated with low IgM titres to modified LDL [15].

In contrast with IgM titres, IgG titres were higher in women with MI and/or diabetes than in controls. This is in agreement with a previous study on both men and women, in which levels of IgG antibodies to MDA-LDL were significantly higher in patients with coronary artery disease and/or diabetes than in controls [40]. However, in other studies IgG titres were not associated with extent of atherosclerosis [16] or carotid intima-media thickness [14,15].

In the present study there was no obvious difference between the three subgroups of patients with regard to antibody titres to modified LDL, which is in accordance with the results of Griffin et al. [40]. Thus diabetic women without prior MI were more similar to women with prior MI (both diabetic and non-diabetic) than to controls. This is in agreement with epidemiological studies in which the risk of diabetic patients without previous MI having a primary MI was as high as that of non-diabetic subjects with previous MI developing a re-infarction [6].

Elevated levels of antibodies against ox-LDL have been reported previously in patients with diabetes [18–21], although some authors have reported divergent results [14,22]. However, those studies included only men, or both genders without a special analysis of women. As CVD is a major threat in women with diabetes, we considered it important to focus on women specifically.

In the present study, use of ACE inhibitors or lipid-lowering drugs was associated with lower IgM and higher IgG titres. However, in regression analyses this was explained by the presence of MI and/or diabetes. Thus medication by itself did not seem to affect antibody titres.

CRP is considered to be a sensitive marker for systemic inflammation. Its concentration is determined mainly by the liver in response to cellular cytokines [23]. Prior studies of the association of CRP levels with CVD have mainly been limited to men. However, CRP was reported to be a strong, independent risk factor for CVD in previously apparently healthy women, with the risk for MI or stroke being 7-fold greater in the highest quartile of CRP [27]. The results of the present study are in accordance with this, as the highest CRP levels were observed in diabetic women with MI, a group with a high risk of recurrent CVD and death. Furthermore, the significantly higher CRP levels among the non-MI diabetic women compared with controls, which probably contribute to an increased future risk of MI [6], call attention to the importance of careful prophylactic measurements in this group of patients.

It is probable that the positive associations of CRP level with BMI, WHR, SBP and triacylglycerols, and its negative association with HDL-cholesterol, found in the present study, and reported previously by others [24,25], do contribute to the increased risk of CVD. The positive association between HbA1c and CRP in the present study might indicate that poor glycaemic control contributes to atherosclerosis not only via glycation but also as a result of increased inflammation. A stronger influence of other factors might explain why CRP level was not associated with smoking in the present study.

In conclusion, we have found that women with diabetes, even without clinical evidence of CHD, had an immune response to ox-LDL which was similar to that of women (with or without diabetes) who had sustained an MI. These women also had elevated CRP levels, which were highest in the group with both MI and diabetes. This group also had the highest WHRs, with concomitant low HDL and high triacylglycerols. The three groups with diabetes, MI or both displayed similar immune responses, in contrast with healthy women.

The present data cannot explain why women with diabetes lose their protection from CHD. However, they might indicate that the atherosclerotic process, with a concomitant inflammatory response, could already be well under way in women with diabetes even in the absence of clinical signs of atherosclerosis. The combination with an adverse risk factor profile may, at least partly, explain the increased morbidity and mortality from CHD in women with diabetes. Both primary and secondary preventative measures are therefore of
considerable importance in efforts to decrease morbidity and mortality among women with diabetes.

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Autoantibodies, C-reactive protein, diabetes and myocardial infarction in women

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