Inflammation-Sensitive Plasma Proteins and Incidence of Myocardial Infarction in Men With Low Cardiovascular Risk

Gunnar Engström, Lars Stavenow, Bo Hedblad, Peter Lind, Patrik Tydén, Lars Janzon, Folke Lindgärde

Objective—Myocardial infarction (MI) is sometimes experienced by individuals without any traditional risk factor. This prospective study explored whether incidence of MI in nonsmoking, nondiabetic men with normal blood pressure and serum lipids is related to inflammation-sensitive plasma proteins (ISPs).

Methods and Results—Five ISPs (α1-antitrypsin, haptoglobin, ceruloplasmin, fibrinogen, orosomucoid) were analyzed in 6075 men, 47±3.6 years old. A low-risk group (no traditional risk factor, n=1108) and a high-risk group (≥2 major risk factors, n=1011) were defined. Incidence of MI (n=227) was monitored over 18.1±4.3 years of follow-up. In the low-risk group, the age-adjusted relative risks (RRs) were 1.00 (reference), 1.9 (95% CI, 0.8 to 4.2), 1.8 (95% CI, 0.6 to 5.4), and 2.9 (95% CI, 1.05 to 8.1), respectively, for men with 0, 1, 2, and ≥3 ISPs in the top quartile (trend: P=0.03). In this group, the increased risk was observed only after ≥10 years of follow-up. In the high-risk group, the age-adjusted RRs were 1.00, 1.4 (95% CI, 0.9 to 2.2), 1.9 (95% CI, 1.2 to 3.1), and 2.0 (95% CI, 1.3 to 3.1), respectively, for men with 0, 1, 2, and ≥3 ISPs in the top quartile (trend: P=0.0004).

Conclusion—Incidence of MI in nonsmoking, nondiabetic men with normal blood pressure and lipids was related to ISPs. The causes for this relationship remain to be explored. (Arterioscler Thromb Vasc Biol. 2003;23:1-5.)

Key Words: myocardial infarction ■ inflammation ■ epidemiology ■ risk factors

Dyslipidemia, hypertension, smoking, and diabetes are well-known risk factors for atherosclerosis and myocardial infarction (MI). Even in prospective studies of presumed healthy subjects, those who later have MI generally are subjects with at least one of these risk factors. However, MI is sometimes experienced by individuals without any major risk factor. Few studies have been specifically performed on this group. During recent years there has been a growing recognition that inflammation plays an important role in the development of cardiovascular diseases. No previous study has explored whether inflammation is associated with incidence of MI in men with low levels of the traditional risk factors.

The Malmö Preventive project was a screening program for detection of individuals with high risk for cardiovascular disease. Measurement of five inflammation-sensitive proteins (ISPs), α1-antitrypsin, haptoglobin, ceruloplasmin, fibrinogen, and orosomucoid, was part of the program for approximately 6000 healthy men. The health examinations were performed between 1974 and 1983. Previous follow-up studies from this cohort have shown that all five ISPs were significantly associated with incidence of MI. It has also been shown that elevated levels of these proteins are associated with increased levels of other risk factors, including diabetes, blood pressure, smoking, and cholesterol, and that elevated ISPs add to the cardiovascular risk associated with these risk factors. The aim of this study was to explore whether a low-grade inflammation is associated with incidence of MI in nonsmoking, nondiabetic men with normal blood pressure and lipids. The relation between inflammation and MI in this low-risk group is compared with the relationship in a high-risk group with at least two major risk factors.

Methods

Between 1974 and 1983, 22444 men participated in a screening program for detection of individuals with high risk for cardiovascular diseases. Participation rate was 71%. The presence of five ISPs (α1-antitrypsin, haptoglobin, ceruloplasmin, fibrinogen, orosomucoid) was determined in 6193 men at the time of the health examination. Men with a history of MI and stroke (according to questionnaire and the Swedish hospital discharge register) were excluded. Men who reported that they had had cancer were also excluded. After the exclusions, 6075 men remained.

Subjects were categorised into nonsmokers and smokers using the question “Are you a smoker?” Cigarette consumption was categorised into daily consumption of up to 10 cigarettes, 11 to 20 cigarettes, and ≥20 cigarettes per day.
TABLE 1. Baseline Characteristics in Men With Low Cardiovascular Risk (No Risk Factor) and Men With High Cardiovascular Risk (≥2 Risk Factors) in Relation to ISP Levels.

<table>
<thead>
<tr>
<th></th>
<th>Low Cardiovascular Risk</th>
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<th>High Cardiovascular Risk</th>
<th></th>
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<tr>
<td></td>
<td>Low ISPs</td>
<td>High ISPs</td>
<td>P*</td>
<td>Low ISPs</td>
<td>High ISPs</td>
<td>P*</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>911</td>
<td>197</td>
<td></td>
<td>504</td>
<td>507</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.4±3.8</td>
<td>46.6±3.7</td>
<td>0.63</td>
<td>46.8±3.5</td>
<td>47.5±3.4</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>0</td>
<td>0</td>
<td></td>
<td>80</td>
<td>91</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>&gt;20 cigarettes/d (% of smokers)</td>
<td>0</td>
<td>0</td>
<td></td>
<td>26</td>
<td>33</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>119±8.4</td>
<td>120±8.3</td>
<td>0.74</td>
<td>141±16</td>
<td>142±18</td>
<td>0.46</td>
<td></td>
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<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>80±5.0</td>
<td>80±5.0</td>
<td>0.93</td>
<td>96±10</td>
<td>97±11</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Treatment for hypertension (%)</td>
<td>0</td>
<td>0</td>
<td></td>
<td>12</td>
<td>14</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.2±0.74</td>
<td>5.1±0.73</td>
<td>0.71</td>
<td>6.1±1.2</td>
<td>6.2±1.2</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L) median (interquartile range)</td>
<td>1.05 (0.6)</td>
<td>1.13 (0.6)</td>
<td>0.08</td>
<td>1.90 (1.5)</td>
<td>1.93 (1.5)</td>
<td>0.97</td>
<td></td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>24.0±2.6</td>
<td>24.4±2.9</td>
<td>0.14</td>
<td>26.2±3.5</td>
<td>26.4±4.0</td>
<td>0.71</td>
<td></td>
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<tr>
<td>Diabetes (%)</td>
<td>0</td>
<td>0</td>
<td></td>
<td>25</td>
<td>22</td>
<td>0.17</td>
<td></td>
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<tr>
<td>Physical inactivity (%)</td>
<td>49</td>
<td>44</td>
<td>0.18</td>
<td>64</td>
<td>63</td>
<td>0.80</td>
<td></td>
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<tr>
<td>Angina pectoris (%)</td>
<td>0</td>
<td>0</td>
<td></td>
<td>2.4</td>
<td>2.2</td>
<td>0.82</td>
<td></td>
</tr>
</tbody>
</table>

*Low and high ISPs are defined as 0–1 or 2–5 ISPs in the top quartile.

Values are mean±SD or %, unless otherwise stated.

*Comparison between the two low-risk groups and between the two high-risk groups.

Blood pressure (mm Hg), in supine position, was measured twice in the right arm after a 10-minute rest. The average of two measurements was used. A sphygmomanometer and a rubber cuff of appropriate size were used.

Physical inactivity was noted for men who reported that they were mostly sedentary in their spare time. Subjects who confirmed a doctor’s diagnosis of angina pectoris or who used nitrates were considered to have angina pectoris.

Men with fasting whole blood glucose ≥6.1 mmol/L and/or 2-hour post-load glucose ≥10.0 mmol/L (glucose load: 30 g per m² body surface area) and men with self-reported diabetes were considered to have diabetes. Serum cholesterol and triglycerides were analyzed with standard methods at the laboratory of the university hospital.

High versus Low Cardiovascular Risk

The group with a low risk for cardiovascular disease was defined as men who were nonsmokers without diabetes, with cholesterol <6.5 mmol/L and triglycerides <2.3 mmol/L, whose systolic and diastolic blood pressures were <140/90 mm Hg, and who were not treated for hypertension. Men who reported a history of angina pectoris (n=7) were excluded from the low-risk group. The cut-off levels for serum lipids were according to the national guidelines for treatment of hyperlipidemia.7,23 The high-risk group was defined as those with at least two of the following risk factors: smoking, diabetes, hypertension (systolic or diastolic pressures ≥160/95 mm Hg or treatment for hypertension), or dyslipidemia (both cholesterol ≥6.5 mmol/L and triglycerides ≥2.3 mmol/L).

Inflammation-Sensitive Plasma Proteins

An electroimmunoassay method was used to assess the plasma levels of five ISPs.22 The analyses were performed consecutively at the time of screening. These proteins were chosen since they are used in clinical practice in order to estimate inflammatory activity. The precision of the analysis had a standard error of <5%.22 The detection limits were 20 mg/L for ceruloplasmin, 50 mg/L for α1-antitrypsin, and 350 mg/L for orosomucoid, haptoglobin, and fibrinogen.

We have shown that the correlation coefficients between the individual proteins range from 0.31 to 0.5622 and that the relationships between ISP and cardiovascular diseases are nonlinear; ie, the risk increases most between the 3rd and 4th quartiles of ISP.22 In accordance with our previous studies,21,22,23 the sample was categorized according to the number of ISPs in the top quartile.

Follow-up

All cases were followed from the baseline examination until death or until December 31, 1997. A cardiac event was defined as fatal or nonfatal MI (code 410 according to the International Classification of Diseases, 9th revision, ICD-9), or death due to chronic ischemic heart disease (ICD-9 codes 412 to 414). The Malmö Myocardial Infarction Register,23 the Swedish Hospital Discharge Register, and the Swedish Causes of Deaths Register were used for case retrieval. Of the 227 cardiac events, 142 were nonfatal. Of the 85 fatal cases, the cause of death was based on autopsy for 58 (68%). A validation study from the Swedish Hospital Discharge Register showed that the diagnosis “myocardial infarction” was false in only 5% of the cases.24

Statistics

The Pearson’s chi-square and the Mantel–Haenszel chi-square tests were used for comparisons of categorical variables between groups. Because of the small number of cardiac events in the low-risk group, the Mann–Whitney U test was used for continuous variables. The Cox’ proportional hazards regression was used for the analysis of the cardiac event rates with adjustments for age and potential confounders; ie, factors that differed between groups with high and low ISPs. The SPSS statistical software (v 8.0) was used for the analysis.25

Results

Subjects

A total of 1108 men had a low cardiovascular risk (no major risk factors), and 1011 had high cardiovascular risk (at least 2 major risk factors). The distribution of risk factors in relation to ISP levels is given in Table 1.

Incidence of Cardiac Events in the Low-Risk Group

Incidence of cardiac events increased with the number of ISPs in the top quartile. In the low-risk group, the age-
adjusted relative risks (RRs) were 1.00 (reference), 1.9 (95% CI, 0.8 to 4.2), 1.8 (95% CI, 0.6 to 5.4), and 2.9 (95% CI, 1.05 to 8.1), respectively, for men with 0, 1, 2, and ≥3 ISPs in the top quartile (P for trend = 0.03). There was no significant difference in cholesterol, blood pressure, or BMI between low-risk individuals with high and low ISPs (Table 1). The triglyceride levels were somewhat higher in the group with high ISPs (1.05 versus 1.13 mmol/L, P = 0.08). However, the relation between cardiac events and number of elevated ISPs was similar after further adjustments for log-transformed triglycerides values (P for trend = 0.050).

In men with low cardiovascular risk, the relationship between ISPs and cardiac events was only observed after more than 10 years of follow-up. Only one man with high ISPs had a cardiac event during the first 10 years. After more than 10 years of follow-up, the relative risks were 1.00, 1.8 (95% CI, 0.7 to 4.8), 2.5 (95% CI, 0.8 to 8.1), and 3.3 (95% CI, 1.04 to 11), respectively, for men with 0, 1, 2 and ≥3 elevated ISPs (P for trend = 0.02).

Figure 1 illustrates the incidence of cardiac events over time in relation to cardiovascular risk and 0 to 1 versus 2 to 5 elevated ISPs. For the first 10 years, the age-adjusted RR in men with low risk and 2 to 5 ISPs was 0.7 (95% CI, 0.1 to 5.4), as compared with low-risk men with 0 to 1 elevated ISPs. After more than 10 years of follow-up, the RR was 2.3 (95% CI, 1.01 to 5.4) for low-risk men with 2 to 5 ISPs (Figure 1).

Incidence of Cardiac Events in the High-Risk Group

In the high-risk group, the age-adjusted RRs were 1.00 (reference), 1.4 (95% CI, 0.9 to 2.2), 1.9 (95% CI, 1.2 to 3.1), and 2.0 (95% CI, 1.3 to 3.1), respectively, for men with 0, 1, 2 and ≥3 ISPs in the top quartile. After adjustments for cholesterol, smoking, and cigarette consumption, the RRs for men with 0, 1, 2, and ≥3 elevated ISPs were 1.00, 1.4 (95% CI, 0.9 to 2.2), 1.9 (95% CI, 1.2 to 3.0), and 1.9 (95% CI, 1.2 to 2.9), respectively (P for trend = 0.002). The relations between MI and number of elevated ISPs were significant both for early events [<10 years, RRs: 1.00, 1.4 (95% CI, 0.6 to 3.1), 2.3 (95% CI, 1.1 to 5.0) and 2.4 (95% CI, 1.2 to 5.0), respectively (P for trend = 0.07)] and late events [≥10 years, RRs: 1.00, 1.4 (95% CI, 0.8 to 2.4), 1.7 (95% CI, 0.96 to 3.1), and 1.7 (95% CI, 1.00 to 3.0), respectively (P for trend = 0.04)]. If anything, the relationships were attenuated after more than 10 years of follow-up.

Characteristics of Men With and Without Cardiac Events

In men with high and low cardiovascular risk, the number of elevated ISPs was higher in men who subsequently had cardiac events (Table 2). Cholesterol and blood pressure were not significantly associated with cardiac events in the low-risk group. As expected, men with a high cardiovascular risk generally had higher ISPs. With the exception of fibrinogen in the low-risk group, the individual ISPs were higher in those who had cardiac events as compared with those who remained free from disease, and the magnitude of the difference was largely similar in the group with low cardiovascular risk as compared with the high-risk group.

Discussion

Some men without traditional risk factors nevertheless have MI. From a scientific point of view, this is an interesting group that has received very little attention.1 This study explored whether MI in nonsmoking, nondiabetic men with normal blood pressure and lipids could be related to ISP levels. The risk of MI in this group increased with the number of elevated ISPs. However, the relationship was only observed after more than 10 years of follow-up.

Cut-off levels for lipids, glucose and blood pressure are often controversial. The cardiovascular risk for the low-risk group would be even lower if lower cut-off levels for cholesterol and blood pressure had been used. This would, however, require a larger cohort with data on ISPs. In our opinion, the cut-off levels have been fully adequate for the purpose of the present study, which was to identify two polar groups with low and high cardiovascular risk. As the traditional risk factors were similar in

Figure 1. Cardiac event-free survival in men with high or low cardiovascular risk in relation to the number of ISPs in the top quartile (0 to 1 versus 2 to 5). Men with low risk and 2 to 5 ISPs had no increased risk during the first 10 years. After more than 10 years of follow-up, this group had a significantly increased risk (see text).
the two low-risk groups, differences in traditional risk factors at baseline cannot explain the increased risk in men with low risk and high ISP levels.

In men with low cardiovascular risk, high ISPs were not associated with MI during the first 10 years, but after that period, elevated ISPs were associated with an increased risk. There could be several explanations for this finding. First, the progression of atherosclerosis could be increased in men with high ISPs, even with low levels of the traditional risk factors.2,26 The long time period to the cardiac events could reflect a slower progression rate in men with low cardiovascular risk. An alternative possibility is that ISPs are unrelated to cardiac events unless other risk factors are also present. It can be assumed that a non-negligible proportion had developed at least one major risk factor after 10 years of follow-up.2,27 Longitudinal studies have shown that incidence of hypertension and diabetes is higher in men with high ISPs compared with those with low ISPs.15,28 The long time period to the cardiac events could reflect the time needed for other risk factors to develop. It is also possible that there had been a significant difference for the first 10 years if the study population had been larger.

In contrast to the low-risk group, men with high cardiovascular risk and high ISPs showed an increased risk during the first 10 years of follow-up and, if anything, the relative risk decreased after that time. Studies of ISPs in the elderly, a group with a high atherosclerotic burden, have shown that high ISPs are associated with incidence of cardiovascular diseases during the first years and far less predictive after several years of follow-up.1,29,30 As the risk was high during the first years, it is speculative but not unreasonable to suggest that ISPs may be related to the occlusive events and not only to an increased progression of atherosclerosis. The ISP levels in high-risk individuals could be associated with the activity of inflammatory cells in the atherosclerotic plaque.31,32

A limitation of the study is that no information on the subfractions of cholesterol was available. Whether the levels of HDL and LDL differed between the groups is not known. However, the results were essentially unchanged when the triglyceride levels were used in the analysis as a proxy for dyslipidemia. Men with hypertension and high cholesterol were referred for further evaluation and treatment.10 These risk factors were similar in both high-risk groups, and it is therefore likely that the benefit of the interventions was similar. Smokers were recommended to stop, but were not offered any help to do so. We do not know whether smoking cessation differed between the high-risk groups.

The ISP levels were associated with incidence of MI in nondiabetic, nonsmoking men with normal blood pressure and serum lipids. This relationship was observed only after more than 10 years of follow-up. The causes for this relationship remain to be explored.

### Acknowledgments

This research was supported by grants from the Swedish Council for Work Life and Social Research, the Åke Wiberg Foundation, and the Apotekare Hedberg Foundation.

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**TABLE 2. Baseline Levels of Cardiovascular Risk Factors in Men With Low Cardiovascular Risk (No Risk Factor) and High Risk (≥2 Risk Factors) in Relation to Cardiac Events During a Mean Follow-up of 18 Years.**

<table>
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<tbody>
<tr>
<td></td>
<td>Cardiac Events</td>
<td>Cardiac Events</td>
<td></td>
<td>Cardiac Events</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>P</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>47.9 ± 3.7</td>
<td>46.4 ± 3.7</td>
<td>0.04</td>
<td>47.7 ± 3.0</td>
</tr>
<tr>
<td><strong>Systolic BP (mm Hg)</strong></td>
<td>121 ± 8</td>
<td>119 ± 8</td>
<td>0.12</td>
<td>145 ± 13</td>
</tr>
<tr>
<td><strong>Diastolic BP (mm Hg)</strong></td>
<td>81 ± 4</td>
<td>80 ± 5</td>
<td>0.58</td>
<td>97 ± 11</td>
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<tr>
<td><strong>Cholesterol (mmol/L)</strong></td>
<td>5.3 ± 0.7</td>
<td>5.2 ± 0.7</td>
<td>0.24</td>
<td>6.4 ± 1.2</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L) median (interquartile range)</strong></td>
<td>1.4 (0.6)</td>
<td>1.05 (0.6)</td>
<td>0.02</td>
<td>2.1 (1.4)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.3 ± 3.2</td>
<td>24.0 ± 2.7</td>
<td>0.07</td>
<td>26.9 ± 4.0</td>
</tr>
<tr>
<td><strong>Smokers (%)</strong></td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>87</td>
</tr>
<tr>
<td><strong>Diabetes (%)</strong></td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>24</td>
</tr>
<tr>
<td><strong>α1-antitrypsin (g/L)</strong></td>
<td>1.3 ± 0.24</td>
<td>1.2 ± 0.24</td>
<td>0.002</td>
<td>1.4 ± 0.27</td>
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<tr>
<td><strong>Ceruloplasmin (g/L)</strong></td>
<td>0.31 ± 0.05</td>
<td>0.30 ± 0.06</td>
<td>0.21</td>
<td>0.34 ± 0.06</td>
</tr>
<tr>
<td><strong>Fibrinogen (g/L)</strong></td>
<td>3.24 ± 0.81</td>
<td>3.25 ± 0.71</td>
<td>0.99</td>
<td>3.9 ± 0.81</td>
</tr>
<tr>
<td><strong>Haptoglobin (g/L)</strong></td>
<td>1.24 ± 0.61</td>
<td>1.13 ± 0.54</td>
<td>0.25</td>
<td>1.67 ± 0.79</td>
</tr>
<tr>
<td><strong>Orosomucoid (g/L)</strong></td>
<td>0.80 ± 0.15</td>
<td>0.74 ± 0.19</td>
<td>0.02</td>
<td>0.91 ± 0.21</td>
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<tr>
<td><strong>No ISP in top Q (%)</strong></td>
<td>42</td>
<td>60</td>
<td>Trend: 0.04</td>
<td>15</td>
</tr>
<tr>
<td><strong>1 ISP in top Q (%)</strong></td>
<td>30</td>
<td>22</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td><strong>2 ISP in top Q (%)</strong></td>
<td>12</td>
<td>10</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td><strong>≥3 ISP in top Q (%)</strong></td>
<td>15</td>
<td>8</td>
<td>37</td>
<td>29</td>
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<tr>
<td><strong>ISP in top Q (n)</strong></td>
<td>1.1 ± 1.2</td>
<td>0.7 ± 1.1</td>
<td>0.03</td>
<td>2.1 ± 1.46</td>
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</table>

Values are mean ± SD or %, unless otherwise stated.
References


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