The risk of myocardial infarction is enhanced by a synergistic interaction between serum insulin and smoking

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Abstract

Objectives: To evaluate the relationship between levels of serum insulin, the homeostasis model assessment (HOMA) and IGF-binding protein-1 (IGFBP-1) as factors related to myocardial infarction (MI) risk, and their interaction with lifestyle-related risk factors.

Design: The Stockholm epidemiology programme (SHEEP), a case-control study, consisting of 749 first-time MI cases (510 men, 239 women) and 1101 healthy controls (705 men, 396 women) was used.

Methods: The risk of developing MI was assessed by calculating odds ratios (OR) and synergistic interactions (SI) between serum insulin, IGFBP-1, HOMA and other variables related to MI risk (including smoking) in men and women.

Results: Subjects with elevated levels of insulin and HOMA (> 75th percentile) had increased MI risks when compared with individuals with low levels. ORs for elevated insulin and HOMA (adjusted for age and residential area) for men: insulin 1.6 (95% confidence interval (CI) 1.3–2.1) and HOMA 1.5 (95% CI 1.1–1.9) and for women: insulin 2.1 (95% CI 1.5–2.9) and HOMA 1.9 (95% CI 1.3–2.8). Women with low levels of IGFBP-1 (> 10th percentile) showed a tendency towards elevated MI risk even if this was not statistically significant (OR 1.5 (95% CI 0.9–2.6)). Smokers with high levels of serum insulin had greatly increased MI risk (OR for men: 4.7 (95% CI 3.0–7.2) and OR for women: 8.1 (95% CI 4.3–14.8)). SI scores based upon these interactions were statistically significant.

Conclusions: These results might have preventive cardiovascular implications as they clearly suggest that subjects with insulin resistance are particularly susceptible to the hazards of smoking.

European Journal of Endocrinology 147 641–647

Introduction

Insulin resistance, desensitisation of the peripheral tissues to the effects of insulin, is associated with a cardiovascular risk pattern referred to as the insulin resistance syndrome (IRS) including elevated blood pressure, serum triglycerides and body weight (particularly of the abdominal type), as well as decreased levels of high density lipoprotein (HDL). Since its discovery during the late 70s and early 80s, IRS has been concluded to be an important risk factor for the development of coronary heart disease (CHD) in several large prospective studies such as the Paris prospective study (1), the Russeton study (2), the Whitehall study (3) and the Helsinki policemen study (4).

Central obesity, insulin resistance, or both, have been proposed as underlying risk factors for IRS and several possible causal links between the cluster of metabolic disturbances have been suggested. It is possible that changes in the levels of one component may initiate the change of levels of another component, such as insulin resistance leading to changes in blood pressure and blood lipid metabolism (5). Furthermore, insulin-like growth factor-1 (IGFBP-1), one of the proteins that bind to circulating insulin-like growth factor (IGF)-I, is negatively correlated to low density lipoprotein (LDL) cholesterol, triglycerides, blood pressure, body mass index (BMI) and insulin resistance and positively correlated to HDL cholesterol (6, 7). All of the above mentioned data provide evidence suggesting a possible role for IGFBP-1 in the network of risk factors that may eventually lead to myocardial infarction (MI). There are no previous studies of this size investigating the relationship between IGFBP-1 and CHD or the relationship between IGFBP-1 and insulin resistance.
As lifestyle-related risk factors such as smoking or physical inactivity might be associated with an increased risk not only for CHD but also for insulin resistance, it is important to partition the confirmation of such lifestyle-related factors and insulin resistance in relation to the development of CHD and also establish how they interact with each other. Information about interactive effects is hence not only necessary for the understanding of IRS disease mechanisms but may also contribute to the scientific bases of coronary vascular disease prevention.

The aim of the present study was to further investigate the role of insulin, homeostasis model assessment (HOMA) and IGFBP-1 as risk markers for MI in relation to IRS among first-time MI cases and healthy controls, and to evaluate their interaction with the lifestyle-related risk factors such as current smoking, physical inactivity and job strain. Differences between men and women with respect to these variables were also studied.

**Subjects and methods**

**Study population**

The Stockholm Heart Epidemiology Programme (SHEEP) is a case-control study designed for epidemiological analysis of several risk factors for MI. Study participants were first-time MI patients and controls enrolled in the SHEEP study previously described in detail elsewhere (8). The study base was comprised of all Swedish citizens aged 45–70 years, with no prior clinically diagnosed MI events, living in Stockholm county. The study was conducted by researchers at the Karolinska Institute in collaboration with all ten emergency hospitals in Stockholm county. The study was approved by the Karolinska Institute approved this study.

All subjects gave written, informed consent.

Cases, identified by hospital records, were men and women admitted for treatment of first-time MI events. Male cases were identified during 1992 to 1993 (2 years) and female cases during 1992 to 1994 (3 years). During the initial 8 months of the study, the upper age limit was set at 65 years but this was increased to 70 years for the remainder of the study period. Inclusion criteria for cases was a diagnosed first-time MI according to clinical criteria accepted by the Swedish Association of Cardiologists in 1991 (9).

One referent per case was randomly selected from the Stockholm County population registers after stratification for age, sex and hospital catchment area (residential area). The ethics committee at the Karolinska Institute approved this study.

**Analysis of materials**

All study participants underwent a physical examination, blood sampling and answered an extensive questionnaire. The present analyses are restricted to those who survived for 28 days after their first MI event without further MI events before blood sampling. Study participants with diagnosed diabetes mellitus, and whose questionnaires were filled out by a close relative, e.g. in cases of fatal MI, were excluded. In total, 510 male and 239 female cases and 705 male and 396 female referent persons were included.

Serum samples were collected following overnight fasting, approximately 3 months after the MI event to allow for a stable metabolic period and stored at −70°C until analyses were performed. Each sample was thawed just prior to analysis and assayed for insulin and IGFBP-1 in a blinded manner to reduce the possibility of bias and to minimise variability between assays. IGFBP-1 concentrations were determined using a radioimmunoassay (RIA) technique according to Póvoa et al. (10). The sensitivity of this assay was 3 µg/l and the intra- and interassay coefficients of variations were 3 and 10% respectively. Serum insulin was measured using commercial RIA kits (RIA 100; Pharmacia, Upplands, Sweden) where the detection limit for the insulin assay was 2 µU/ml and the within-assay coefficient of variation was 5.0–5.7%.

An estimate of insulin resistance was calculated using HOMA as follows: insulin resistance = fasting glucose × fasting insulin/22.5 (11). This was calculated for all subjects where both insulin and glucose levels were available; in total, 1093 men (455 cases and 638 controls) and 577 women (215 cases and 362 controls).

**Data analysis**

Men and women were analysed separately. Subjects with levels above limits set by the 75th and 90th percentile levels of insulin and HOMA from the referents were classified as exposed for those variables. As low levels of IGFBP-1 have previously been associated with insulin resistance and hyperinsulinaemia, IGFBP-1 levels below the 25th and 10th percentile levels were classified as exposed. Information about exposure to other risk factors was obtained from questionnaires, anthropometric tests or blood samples from each individual. Subjects receiving anti-hypertensive drug therapy during the data collection or previously or with a systolic blood pressure (BP) ≥ 160 mmHg or diastolic BP ≥ 90 mmHg were classified as hypertensives. Hypercholesterolaemia (≥ 6.5 mmol/l), hypertriglyceridaemia (≥ 2.3 mmol/l) and high LDL/HDL quotient (≥ 4.0) were diagnosed according to clinical criteria. Subjects with BMI > 28 kg/m² (cut-off value corresponding to the 75th percentile among the control group) were classified as being overweight. Smoking habits, physical activity, the use of betablockers or diuretic agents, fibre intake and job strain was based on information obtained from the questionnaire. Former smoking was defined as having smoked daily but having stopped more than 2 years ago and current smoking.
smokers were defined as persons who smoked or had stopped smoking within the last 2 years. Subjects who reported inactive leisure time were defined as physically inactive and job strain was determined from the ratio between psychosocial demands and decision latitude according to the Karasek–Theorell questionnaire (12).

Statistics

Baseline demographic variables were expressed as percentages or medians as appropriate. Differences between medians were analysed using the Wilcoxon test for continuous variables and χ² analysis for categorical variables and correlation between variables were calculated using the Pearson correlation coefficient.

Odds ratios (OR) with 95% confidence intervals (CI) were calculated for insulin and HOMA at the 75th and the 90th percentile levels, using the population with insulin and HOMA levels below these set exposure limits as the unexposed reference group. Similarly, subjects with IGFBP-1 levels below the 10th or 25th percentile limit were classified as exposed and subjects above these limits were classified as unexposed for OR calculations. OR, including adjustments for potential confounders were calculated using logistic regression. Crude measurements were adjusted for age and residential area. In addition, one set of analysis adjustments was made for physical inactivity, current and former smoking, job strain, betablockers, diuretics and fibre intake. A second set of adjustment analyses included these, as well as hypertriglyceridaemia. BMI > 28, LDL/HDL ratio ≥ 4.0 and BP ≥ 160/90.

Biological interaction between two variables was calculated using the synergy index (SI) score with 95% CI based on the ratio of the combined effects to the sum of the separate effects of two variables according to Rothman & Greenland (1998) (13). An SI score ≠ 1 indicates a departure from an additive effect between two variables, i.e. a significant SI score above one indicates that synergy exists between two variables. SI scores were calculated for the 75th percentile level of exposure for insulin and HOMA and the 25th percentile level for IGFBP-1. These calculations were adjusted for age, residential area, betablockers, diuretics, job strain and fibre intake. The SAS system for Windows versions 6.11 and V8 were used for all statistical and epidemiological analyses (SAS Institute Inc.).

Results

Table 1 describes the baseline characteristics and cut-off limits for classification of exposure for insulin and HOMA (75th and 90th percentiles) as well as IGFBP-1 among cases and referents. The values in italics indicate the percentile cut-off limits used to define exposure.

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases n</td>
<td>Ctrl n</td>
</tr>
<tr>
<td>Age (median years)</td>
<td>59</td>
<td>814</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>52.2</td>
<td>31.4</td>
</tr>
<tr>
<td>Former smokers (%)</td>
<td>28.1</td>
<td>33.3</td>
</tr>
<tr>
<td>Physical inactivity (%)</td>
<td>43.1</td>
<td>31.3</td>
</tr>
<tr>
<td>Job strain (%)</td>
<td>25.2</td>
<td>21.2</td>
</tr>
<tr>
<td>Overweight (% BMI &gt; 28)</td>
<td>30.6</td>
<td>21.8</td>
</tr>
<tr>
<td>Hypercholesterolaemia (% &gt; 6.5 mmol/l)</td>
<td>38.0</td>
<td>26.6</td>
</tr>
<tr>
<td>High LDL/HDL (% &gt; 4.0)</td>
<td>56.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Hypertriglyceridaemia (% &gt; 2.3 mmol/l)</td>
<td>27.8</td>
<td>13.8</td>
</tr>
<tr>
<td>Hypertension (% &gt; 160/90)</td>
<td>25.7</td>
<td>20.9</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>Median 10</td>
<td>510</td>
</tr>
<tr>
<td>75th percentile level</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>90th percentile level</td>
<td>23.5</td>
<td>18</td>
</tr>
<tr>
<td>HOMA</td>
<td>Median 2.1</td>
<td>455</td>
</tr>
<tr>
<td>75th percentile level</td>
<td>3.3</td>
<td>2.8</td>
</tr>
<tr>
<td>90th percentile level</td>
<td>5.9</td>
<td>4.4</td>
</tr>
<tr>
<td>IGFBP-1 (µg/l)</td>
<td>Median 21</td>
<td>510</td>
</tr>
<tr>
<td>10th percentile level</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>25th percentile level</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

* P < 0.05.
P values are for cases vs referents.
of IGFBP-1 between cases and controls but men had lower median serum levels than women (21 μg/l for male controls and 29 μg/l for female controls, \(P < 0.001\)). IGFBP-1 was found to be negatively correlated with insulin and these correlations were not significantly different between cases and controls (men: −0.22 cases and −0.21 controls; women: −0.27 cases and −0.27 controls, \(P < 0.0001\)) and HOMA (men: −0.16 cases and −0.16 controls; women: −0.23 cases and −0.19 controls, \(P < 0.0001\)).

Table 1 also shows the differences between cases and controls with regard to other risk markers commonly associated with MI risk (smoking, physical inactivity, excess weight, hypercholesterolaemia, high LDL/HDL quotient, hypertriglyceridaemia and hypertension).

The exposure of high insulin levels at the 75th and the 90th percentile levels was significantly associated with the risk of developing MI for men and women (shown in Table 2) with ORs being similar at both levels of exposure and for both sexes. HOMA values showed the same pattern as for insulin. The associations of insulin and HOMA to MI were slightly lower after adjustments for current and former smoking, job strain, physical inactivity, beta-blockers, diuretics and fibre intake. When further adjustments for components of the metabolic syndrome (hypertriglyceridaemia, BMI \(>28\), LDL/HDL ratio \(>4.0\) and BP \(>160/90\)) were made, ORs were not significant (data not shown).

Women with IGFBP-1 levels below the 10th percentile tended towards an increased MI risk (adjusted OR = 1.6 (95% CI 0.9–2.8)). However, since the CI values ranged across 1.0, this finding was not statistically significant. No significant association with MI risk was observed among men exposed to low levels of IGFBP-1. The possibility of a risk gradient existing between different levels of IGFBP-1 and MI was assessed by comparing three levels of exposure (<10th percentile, 10th–25th percentile, 25th–75th percentile limits) to one reference group (>75th percentile limit). No such risk gradient was detected however (data not shown).

As illustrated in Fig. 1, exposure to both smoking and high levels of serum insulin infers an even greater risk than insulin or smoking alone, for both men and women (men: OR 4.7 (95% CI 3.0–7.2); women: OR 8.1 (95% CI 4.5–14.8)). The risk estimates for the combined exposure of smoking and HOMA are slightly smaller, but the trend is the same as for the insulin findings. The exact OR (95% CI) illustrated in Fig. 1 are also presented in Table 3 with the results from the interaction analyses. Significant SI was found between current smoking and insulin (men: SI 2.6 (95% CI 1.2–5.6); women: SI 3.0 (95% CI 1.3–6.9)) and between current smoking and HOMA (men: SI 2.1 (95% CI 1.0–4.5); women: SI 2.9 (95% CI 1.2–7.1)). Individuals exposed to low levels of IGFBP-1 and current smoking had an increased MI risk (men: OR 3.4 (95% CI 2.1–5.4); women: OR 5.3 (95% CI 2.8–10.2)). Even though these were not statistically significant, SI scores for interaction between low levels of IGFBP-1 and current smoking (men: SI 1.7 (95% CI 0.7–3.7); women: SI 2.5 (95% CI 0.9–6.9)) indicate that an SI might exist. No significant interactions were found between former smoking and elevated insulin, HOMA or low IGFBP-1. Also, the OR for the combined exposure of former smoking and insulin (men: OR 1.4 (95% CI 3.0–7.2); women: OR 1.9 (95% CI 0.9–3.9)) were much lower than the corresponding figures for current smoking, clearly indicating the benefits of stopping smoking. Physical inactivity was shown not to significantly interact with insulin, HOMA or IGFBP-1.

### Discussion

The salient findings of our study were that a synergistic interactive effect exists between insulin resistance and smoking in relation to MI risk, with ORs for persons exposed to both variables being significantly greater than the additive effects from the risks imposed by the two variables alone. Furthermore, it confirmed previous reports on the significant risk-increasing effects of serum insulin and HOMA (as markers of insulin resistance) on MI in both men and women.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Insulin (OR (95% CI))</th>
<th>HOMA (OR (95% CI))</th>
<th>IGFBP-1 (OR (95% CI))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;75th</td>
<td>&gt;90th</td>
<td>n</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude*</td>
<td>1.6</td>
<td>1.6</td>
<td>1214</td>
</tr>
<tr>
<td>Adjusted†</td>
<td>1.3</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude*</td>
<td>2.1</td>
<td>2.2</td>
<td>633</td>
</tr>
<tr>
<td>Adjusted†</td>
<td>2.0</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted for age and residential area.
† Adjusted for physical inactivity, current and former smoking, betablockers, diuretics, fibre intake, job strain, age and residential area.
Insulin resistance, smoking and MI risk

In accordance with the prospective epidemiological studies referred to in the Introduction, our data have shown that insulin resistance is accompanied by a significantly increased risk for the development of MI. A similar effect was noted from exposure of low levels of IGFBP-1 (albeit not statistically significant) which was also shown to correlate with insulin levels and HOMA. These latter results are in agreement with recent findings by Ruotolo et al. (14) who reported significant correlations between IGFBP-1 and insulin resistance. However, the males in that study all had low levels of IGFBP-1 and a history of MI. We also observed that differences in median levels of IGFBP-1 and the OR (10th percentile) are slightly higher in women than men. A recent study by Söderberg et al. (15) showed similar results with regard to IGFBP-1 levels, further supporting these findings. Like the Bedford survey of CHD (16), our data indicated no gender differences between serum levels and MI risk inferred by high levels of serum insulin and HOMA.

Perhaps surprisingly, most of the significant ORs persisted after adjustments for the confounding variables of smoking, job strain, physical inactivity, use of beta-blockers or diuretics and fibre intake, all of which are thought to be important factors influencing the risk of developing several factors affecting cardiovascular health. The lack of the expected reduction in the OR from these adjustments indicates that insulin resistance is a risk marker independent from such lifestyle-related influences. In particular, we would have expected physical activity to influence OR since, in several studies, it has been shown to strongly influence both the development of MI and insulin resistance itself. The close relationship between insulin resistance and components of the metabolic syndrome was indicated as OR approached 1 after the inclusion of such variables as hypertriglyceridaemia, hypertension, excess weight, abdominal adiposity (high waist/hip ratio) and disrupted LDL/HDL balance in the logistic regression model. The disappearance of the significant risks are indirect evidence that insulin resistance, BMI etc. are causally related, e.g. in the metabolic syndrome. As these variables are causally related we have not considered them as confounders and they have therefore not been included in the OR or SI calculations.

Smoking has previously been demonstrated to both actively impair insulin action and lead to insulin resistance (17) and MI (previously described in the SHEEP study by Reuterwall et al. (18)). The authors of the former study suggested that this association was not due to the effects of nicotine since using snuff did not elicit insulin resistance. Contradictory to these findings are the results of Eliasson et al. (18), who found that the use of nicotine gum was associated with insulin resistance. Whatever the mechanism might be, the lack of synergism between former smoking and elevated insulin levels provides supportive evidence for the clinical benefits of stopping smoking. In theory, our findings show that by doing so, a woman with elevated serum insulin levels may reduce the risk of developing MI from an odds of 8.1 (exposure to current smoking and high insulin levels) to an odds of 1.9 (former smoking and high insulin). Possible explanations for the synergistic effect could be due to smoking and insulin resistance affecting different physiological mechanisms contributing to MI risk as separate components of the same sufficient cause (e.g. smoking influencing the coagulation cascade and insulin resistance affecting blood lipids). These complex relationships must be further evaluated in order to understand the biological mechanisms behind the relationship between insulin resistance and smoking and their interaction.

As the SHEEP study is of a retrospective case-control design, the data on metabolic factors may not truly reflect the exposure before disease onset. However, blood sampling was carried out at least 3 months after the MI event, at which time metabolic stability should have been regained (19, 20). This is supported by the levels of insulin, HOMA and IGFBP-1 reported here being similar to baseline levels of other prospective studies previously referred to in this paper. Any influence of the MI event itself on metabolic factors must also be regarded as a potential source of error. MI cases are commonly advised on preventive changes in lifestyle, altering smoking habits and diet, and these may influence both insulin resistance and IGFBP-1 status, which could potentially result in an underestimation of the MI risk. Fibre intake as an estimate of the quality of the diet was used in the adjustments to

Figure 1 The risk of developing MI is greatly enhanced in smokers with high serum insulin levels (above 12 μU/ml for men and above 11 μU/ml for women; limits corresponding to the 75th percentile of the control group), especially in women. Non-smokers and those who also had low insulin levels were considered to be at no risk (i.e. OR = 1). The figure illustrates the significant interaction between smoking and high levels of serum insulin where exposure to both factors results in an effect more than twice the sum of the risk inferred by smoking or insulin resistance alone. The exact ORs including 95% CI values and SI scores are presented in Table 3.
reduce such confounding effects. Also, it has been suggested that the increased use of betablockers and diuretics can lead to changed IGFBP-1 levels (21) and induction of insulin resistance (22, 23). The adjustment for these drugs resulted in a slight decrease in the OR. Other sources of error for the risk estimates in this study are based on the information obtained from the questionnaire (such as smoking or physical activity), but such recall bias was minimised by the exclusion of study subjects who were unable to answer the questionnaire themselves.

This study supports the theory that insulin resistance is an important risk marker for MI and that a synergistic effect between smoking and insulin resistance further enhances this association. The results might have implications for cardiovascular prevention as they clearly suggest that persons with insulin resistance may be particularly susceptible to the hazards of smoking.

**Acknowledgements**

This study was supported by the Swedish Medical Research Council, grants numbers 09533 and 04224, the Swedish Heart and Lung Foundation, Ragnar and Tore Söderbergs stiftelse, King Gustaf V and Queen Victoria’s foundation and by the National Network in Cardiovascular Research, Foundation for Strategic Research grant B433 4327/98. The authors would like acknowledge the skilful help of Inga-Lena Wivall and Ella Wallerman (technical assistance) and Gunnar Gräbers (SAS programming).

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Received 19 December 2001
Accepted 16 July 2002