Hormones, haemostasis, and the risk of thrombosis

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Chapter 16

The relation between prolactin, acute stress, and inflammation in patients with myocardial infarction

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Submitted for publication
**Abstract**

**Introduction:** Prolactin may contribute to an atherogenic phenotype, and prolactin secretion increases in acute stress and inflammation. We therefore aimed to investigate the relation between prolactin, acute stress, and inflammation in patients with myocardial infarction.

**Methods:** We performed a case-control study in 40 patients with myocardial infarction and 39 controls, aged 41-84 years. Blood for assessment of prolactin and high sensitive C-reactive protein (hsCRP) was drawn at inclusion, i.e. during the acute phase of the event, and several weeks hereafter.

**Results:** Prolactin levels at inclusion did not differ between cases and controls (7.0 ng/mL and 6.0 ng/mL, respectively, \( p = 0.28 \)). Two to three weeks later prolactin levels in cases had not decreased. However, univariate regression analysis indicated that hsCRP is associated with prolactin levels (regression coefficient \( \beta = 0.11; 95\% \text{ CI } 0.01-0.21 \)) in cases during the acute phase of myocardial infarction.

**Conclusions:** These findings indicate that prolactin is involved in the systemic inflammatory response which takes place during myocardial infarction; however, this association is not strong enough to induce higher prolactin levels in patients with myocardial infarction.

**Introduction**

Quite a few hormonal dysbalances have been associated with cardiovascular disease\(^2\). The principal function of the pituitary hormone prolactin is to warrant lactation\(^2\). Nonetheless, recent data indicate that prolactin could contribute to an atherogenic phenotype: hyperprolactinaemia has been associated with impaired endothelial function, dyslipidaemia, hypercoagulability, decreased insulin sensitivity, and low-grade inflammation as represented by high sensitive C-reactive protein (hsCRP)\(^3-6\). Additionally, physiological prolactin levels were found to correlate with a risk score that predicts 10-year cardiovascular mortality\(^7\). The hypothesis that prolactin may play a role in the development of atherothrombotic disease is further supported by clinical data showing higher prolactin levels in patients with myocardial infarction than in patients with unstable angina\(^8\) or in healthy controls\(^8-10\). These studies suggest that prolactin may affect platelet function or the coagulation cascade, thereby influencing thrombosis\(^11\).

However, serum prolactin levels transiently rise after several forms of acute stress, since environmental stressors potentiate production of prolactin and other pituitary-dependent hormones\(^11\). The elevated prolactin levels (still within or just above the upper limit of the physiological range) as observed in patients with myocardial infarction\(^8-10,12-14\) may therefore reflect an increased neuro-endocrine stress response during the acute phase of the event, with or without an additional effect of prolactin on the atherothrombotic process. Furthermore, recent evidence demonstrates that there is bidirectional communication between the neuro-endocrine and immune systems, with pro-inflammatory cytokines exerting a stimulatory effect on local or systemic prolactin production, whilst prolactin in return modulates the immune response\(^11;15;16\). As such, the stress hormone prolactin could be enhanced in inflammatory conditions, for instance in the acute phase of myocardial infarction. We therefore hypothesised that prolactin levels are only temporarily increased in patients with myocardial infarction, as a result of either the acute neuroendocrine stress response or the systemic inflammatory response.

To investigate the relation between prolactin levels, acute stress, and inflammation, we determined levels of prolactin and hsCRP in patients with myocardial infarction, both during the acute phase of the event and several weeks hereafter, and we compared these with levels in gender- and age-matched controls.

**Methods**

**Study design and participants**

The present analysis was part of a case-control study on the association of viral respiratory tract infection and the risk of myocardial infarction. For this study, consecutive patients with proven myocardial infarction were recruited at the coronary care unit (CCU) of the Slotervaart Hospital, Amsterdam, the Netherlands between January 7th, 2008 and January 30th, 2009. Myocardial infarction was defined as enzyme elevation (levels of creatine kinase (CK) twice normal and troponin above normal level) in combination with characteristic ischaemic chest pain, pain in associated referral areas, or ECG changes indicating ischemia\(^17\). Inclusion had to take place within the first 72 hours after presentation. Controls were patients from
the outpatient clinic of the Department of Internal Medicine of the Slotervaart Hospital and were matched to case-patients according to age (within 5 years of the date of birth of the corresponding case-patient) and gender. Controls with a cardiovascular event in the preceding 12 months or symptoms of infection in the previous 2 weeks were excluded. Briefly, during the baseline survey, data on medical history, age, cardiovascular risk factors (smoking, dyslipidaemia, hypertension, diabetes mellitus, and family history), and use of medication were registered in a standardised fashion. The study was performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/ Good Clinical Practice; the study was approved by the Slotervaart Hospital Ethics Committee and all participants gave signed informed consent.

Biochemical analysis
Non-fasting venous blood samples were taken by venipuncture, at baseline and 2 to 3 weeks later. Serum levels of prolactin were measured with chemiluminesometric technology (ADVIA Centaur immunoassay system, Siemens Healthcare Diagnostics, Marburg, Germany). Serum levels of hsCRP were measured with a Near Infrared Particle Immunoassay (SYNCHRON Systems CAL 5 Plus, Beckman Coulter Ireland Inc., Ireland). Samples were analysed in random order and researchers and laboratory personnel had no access to identifiable information.

Statistical analysis
Circulating levels of prolactin and hsCRP had a skewed distribution and were therefore presented as medians with interquartile range (IQR). The Mann-Whitney test was used to compare prolactin and hsCRP levels at inclusion between cases and controls. Since patients were included within the first 72 hours after presentation, additional analysis was performed to compare prolactin levels between cases included at the first, second, and third day, and controls using the Kruskal-Wallis test. The Wilcoxon test was used to compare prolactin and hsCRP levels within groups in time. Additionally, we calculated absolute changes per parameter for each individual by subtracting the values at follow-up from the values at inclusion. Subsequently, medians (interquartile range) of these individual changes were computed, and between-group comparison was performed using the Mann-Whitney test.

Associations between prolactin and hsCRP were assessed using univariate linear regression after log-transformation of both variables. In addition, we evaluated whether troponin or CK-MB as indicators of infarct size could predict prolactin levels using univariate linear regression after log-transformation of all three variables. The results on levels of prolactin and hsCRP are summarised in Table 2. Prolactin levels at inclusion (t=0) did not differ between patients with myocardial infarction and controls; median prolactin level 7.00 ng/mL (interquartile range (IQR) 5.0 to 12.0 ng/mL) and 6.0 ng/mL at inclusion (t=0) did not differ between patients with myocardial infarction and controls; regression coefficient $\beta_{0.11}$ (95% CI 0.01 to 0.21). This association between hsCRP and prolactin levels was not substantially affected by the day of inclusion was taken into account; median prolactin levels of cases included on the first (n=4), second (n=18), and third day (n=18) were 5.5 (IQR 4.0 to 10.8) ng/mL, 8.0 (IQR 5.8 to 10.5) ng/mL, and 6.5 (IQR 5.0 to 15.0) ng/mL, respectively ($p=0.54$). In addition, no difference was observed between prolactin levels at inclusion and follow-up within groups (Table 2 and Figure 1).

Similar results were found in the analysis for men and women separately (Table 2). Exclusion of subjects on prolactin modifying drugs and of subjects with subclinical hypothyroidism did not substantially affect the results (data not shown).

As expected, hsCRP levels at inclusion were higher in cases than controls. CRP levels decreased in cases during follow-up. Linear regression analysis showed a significant relation between levels of hsCRP and prolactin in cases at the moment of myocardial infarction; regression coefficient $\beta_{0.11}$ (95% CI 0.01 to 0.21). This association between hsCRP and prolactin levels was statistically significant in men (regression coefficient $\beta_{0.14}$; 95% CI 0.01 to 0.26), whereas no

### Table 1. Patient characteristics at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=40)</th>
<th>Controls (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years, mean (range)</strong></td>
<td>66.4 (41.83)</td>
<td>65.4 (44.84)</td>
</tr>
<tr>
<td><strong>Male, n (%)</strong></td>
<td>29 (72.5%)</td>
<td>29 (74.4%)</td>
</tr>
<tr>
<td><strong>BMI, kg m⁻², mean (range)</strong></td>
<td>27.4 (24.3;30.0)</td>
<td>26.8 (20.2;35.8)</td>
</tr>
<tr>
<td><strong>Current smoker, n (%)</strong></td>
<td>15 (37.5%)</td>
<td>9 (23.1%)</td>
</tr>
<tr>
<td><strong>Past smoker, n (%)</strong></td>
<td>14 (35.0%)</td>
<td>17 (43.6%)</td>
</tr>
<tr>
<td><strong>Diabetes, n (%)</strong></td>
<td>6 (15.0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Hypertension, n (%)</strong></td>
<td>18 (45.0%)</td>
<td>12 (30.8%)</td>
</tr>
<tr>
<td><strong>Dyslipidaemia, n (%)</strong></td>
<td>20 (50.0%)</td>
<td>8 (20.5%)</td>
</tr>
<tr>
<td><strong>Positive family history, n (%)</strong></td>
<td>20 (50.0%)</td>
<td>10 (25.6%)</td>
</tr>
<tr>
<td><strong>History of CVD, n (%)</strong></td>
<td>19 (47.5%)</td>
<td>1 (2.6%)</td>
</tr>
</tbody>
</table>

The results on levels of prolactin and hsCRP are summarised in Table 2. Prolactin levels at inclusion (t=0) did not differ between patients with myocardial infarction and controls; median prolactin level 7.00 ng/mL (interquartile range (IQR) 5.0 to 12.0 ng/mL) and 6.0 ng/mL (IQR 5.0 to 9.0 ng/mL), respectively, $p=0.28$. Results did not significantly alter when the day of inclusion was taken into account; median prolactin levels of cases included on the first (n=4), second (n=18), and third day (n=18) were 5.5 (IQR 4.0 to 10.8) ng/mL, 8.0 (IQR 5.8 to 10.5) ng/mL, and 6.5 (IQR 5.0 to 15.0) ng/mL, respectively ($p=0.54$). In addition, no difference was observed between prolactin levels at inclusion and follow-up within groups (Table 2 and Figure 1).

Similar results were found in the analysis for men and women separately (Table 2). Exclusion of subjects on prolactin modifying drugs and of subjects with subclinical hypothyroidism did not substantially affect the results (data not shown).

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Prolactin, acute stress, and inflammation in myocardial infarction

A clear association was observed in women (regression coefficient $\beta = 0.08; 95\% \text{ CI} -0.15$ to $0.32$). No relation between hsCRP and prolactin was found in controls, or in cases during follow-up. Linear regression analysis indicated that neither troponin, nor CK-MB, predicts prolactin levels at the moment of myocardial infarction; regression coefficient $\beta = 0.01$ (95% CI -0.11 to 0.12) for troponin, and $-0.03$ (95% CI -0.17 to 0.11) for CK-MB.

Figure 1. Absolute change in prolactin levels per individual. Each bar represents one individual.

Discussion

In contrast to earlier reports\cite{8-10;12-14}, in our study prolactin levels were not higher in patients with myocardial infarction than in controls. Subsequently, no difference was observed between prolactin levels during the acute phase of the event and in the weeks hereafter.

Nevertheless, a positive association between hsCRP and prolactin levels was observed in patients with myocardial infarction during the acute phase of the event, whilst this association was not found in cases during follow-up or in controls, i.e. in situations with less marked inflammatory activity. These findings support the hypothesis that prolactin may be involved in the systemic inflammatory response which takes place during myocardial infarction. However, the contribution of inflammation to circulating prolactin levels appears to be fairly small insofar that even the pronounced inflammatory response during the acute phase of myocardial infarction did not result in higher median prolactin levels.

All together, our results indicate that stress and/or inflammation during the thrombotic event do not substantially affect prolactin secretion in patients with myocardial infarction. Although prolactin levels are only modestly affected by increased inflammatory activity, the immune response itself may still be modulated by prolactin levels.

In contrast to earlier reports\cite{8-10;12-14}, in our study prolactin levels were not higher in patients with myocardial infarction than in controls. Subsequently, no difference was observed between prolactin levels during the acute phase of the event and in the weeks hereafter.

Furthermore, a positive association between hsCRP and prolactin levels was observed in patients with myocardial infarction during the acute phase of the event, whilst this association was not found in cases during follow-up or in controls, i.e. in situations with less marked inflammatory activity. These findings support the hypothesis that prolactin may be involved in the systemic inflammatory response which takes place during myocardial infarction. However, the contribution of inflammation to circulating prolactin levels appears to be fairly small insofar that even the pronounced inflammatory response during the acute phase of myocardial infarction did not result in higher median prolactin levels.

All together, our results indicate that stress and/or inflammation during the thrombotic event do not substantially affect prolactin secretion in patients with myocardial infarction. Although prolactin levels are only modestly affected by increased inflammatory activity, the immune response itself may still be modulated by prolactin levels.

Our findings do not imply that prolactin is not involved in the pathogenesis of atherothrombosis. Although prolactin levels are only modestly affected by increased inflammatory activity, the immune response itself may still be modulated by prolactin levels.

In contrast to earlier reports\cite{8-10;12-14}, in our study prolactin levels were not higher in patients with myocardial infarction than in controls. Subsequently, no difference was observed between prolactin levels during the acute phase of the event and in the weeks hereafter.

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All together, our results indicate that stress and/or inflammation during the thrombotic event do not substantially affect prolactin secretion in patients with myocardial infarction. Although prolactin levels are only modestly affected by increased inflammatory activity, the immune response itself may still be modulated by prolactin levels.
even within the physiological range, thereby enhancing the risk of atherothrombosis\cite{14,19}. This may especially be true since prolactin receptors have been found to be prevalent in human coronary artery plaques\cite{20}. However, the design of our study did not enable us to further disentangle this complex relation between prolactin and inflammation, or any other determinants of atherothrombosis.

Several limitations should be addressed. Our study was not primarily designed to investigate prolactin levels in patients with myocardial infarction. Therefore, blood samples which were used to characterise individuals with respect to prolactin levels were not taken on a specific time point during the day. This may have resulted in a random measurement error because of the diurnal variation in prolactin levels. However, owing to the design of our study, this would have equally affected controls. Furthermore, blood was withdrawn within the first 72 hours after presentation with myocardial infarction and not specifically in the first hours of the event. However, no differences in prolactin levels were observed between patients who were included on the first day of their myocardial infarction and patients who were included on the following days. In addition, Weizman et al showed that prolactin levels were still increased three days after myocardial infarction, and it is therefore unlikely that this delay would have affected our findings\cite{14}. Nonetheless, their results are contradicted by our present data. Finally, the association between prolactin and hsCRP was not statistically significant in women. Since there were only eleven women with a myocardial infarction, this is likely due to a lack of statistical power; the confidence intervals of the regression coefficient by our present data. Finally, the association between prolactin and hsCRP was not statistically significant in women. Since there were only eleven women with a myocardial infarction, this is likely due to a lack of statistical power; the confidence intervals of the regression coefficient are wide enough to encompass a range of effect sizes. In summary, our findings should be interpreted with caution due to the limited sample size.

Overall, our findings provide further insights in the course of prolactin levels after the stressful event of myocardial infarction and its relation to systemic inflammation. We suggest that additional research is needed to determine whether there is a role for prolactin in atherothrombotic disease.

Acknowledgement

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References