Decreased prolactin response to hypoglycaemia in patients with rheumatoid arthritis: correlation with disease activity
Agnes MM Eijsbouts, Frank HJ van den Hoogen, Roland FJM Laan, Fred CGJ Sweep, Ad RMM Hermus, and Leo BA van de Putte

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Agnes M.M. Eijsbouts MD
Frank H.J. van den Hoogen MD, PhD
Roland F.J.M. Laan MD, PhD
Fred C.G.J. Sweep* PhD
Ad R.M.M. Hermus** MD, PhD, professor of endocrinology
Levinus B.A. van de Putte MD, PhD, professor of rheumatology

Departments of Rheumatology, Chemical Endocrinology* and Endocrinology**, University Medical Center Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands

Correspondence to:
Agnes Eijsbouts, Department of Rheumatology, University Medical Center Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.
Telephone: +31-24-3659336
E-mail: a.eijsbouts@maartenskliniek.nl
ABSTRACT

Objective: To compare basal and stimulated prolactin levels between patients with rheumatoid arthritis (RA) and healthy controls (HC) and to assess the effects of antirheumatic treatment on prolactin levels in patients with RA.

Methods: Serum prolactin levels were assessed under basal conditions and during an insulin tolerance test (ITT) in 20 patients with recently diagnosed active RA and 20 age and sex matched HC. The patients with recent onset RA were reassessed after 2 weeks treatment with naproxen and after 6 months of additional treatment with either sulphasalazine or methotrexate. Disease activity was assessed with the disease activity score (DAS).

Results: Basal levels of prolactin were not significantly different between patients with RA and HC. Prolactin responses to hypoglycaemia were significantly lower in untreated patients with RA than in HC. The DAS scores correlated negatively with the area under the curve (AUC) for prolactin levels during the ITT. Treatment with naproxen for 2 weeks did not influence either basal or stimulated prolactin levels. After 6 months of antirheumatic treatment prolactin responses to hypoglycaemia increased significantly to levels observed in HC. At the same time point DAS had improved considerably. This improvement correlated significantly with the increase in AUC of prolactin during the ITT (r=0.48; p=0.05).

Conclusion: Patients with active RA have decreased prolactin responses to hypoglycaemia induced stress. These responses restore following treatment with antirheumatic drugs.

Key words: prolactin, rheumatoid arthritis, NSAIDs, naproxen and antirheumatic treatment.
Prolactin is a hormone, produced by the anterior pituitary gland, which is well known by its ability to stimulate lactation, but it has also been shown to play a role in regulating immune function and has potent pro-inflammatory effects [1,2]. Secretion of prolactin is restrained by the hypothalamus, where the most important prolactin inhibiting factor (PIF), dopamine, is produced. Other factors are also involved in prolactin secretion including proinflammatory cytokines. Depending on the animal species studied and the severity of inflammation, proinflammatory cytokines can either stimulate or depress pituitary prolactin release [3,4]. Prolactin levels are higher in women than in men, though there is considerable overlap in the ranges. Prolactin levels rise in response to all kinds of stress. It has been suggested that excessive prolactin secretion may contribute to the pathogenesis of RA [5]. A characteristic feature of RA is remission of the disease during gestation and exacerbation in the post partum period. During pregnancy prolactin levels are low, but start to rise during the second trimester, preparing the breasts for lactation, and reach their peak at the end of pregnancy, which has been related to the post partum exacerbation of RA. Furthermore, a higher incidence of RA development has been found in the post partum period, particularly when the mother breast feeds [6,7].

Previous studies of prolactin levels in RA patients have shown contradictory results. Prolactin levels have been either increased [5,8-10], decreased [11-14] or unchanged [15-20]. Several explanations may be offered to explain the different results in the literature. Firstly, it may be that NSAIDs influence prolactin levels. In healthy subjects, treatment with NSAIDs can result in increased [21,22], decreased [23,24] or unchanged prolactin levels [25-26]. However, the effects of NSAIDs on prolactin levels have not been studied in patients with RA and in most previous studies on prolactin levels in patients with RA, patients have been examined while using NSAIDs. Secondly, treatment with glucocorticoids may influence prolactin levels. This is suggested by the results of a study by Mateo et al. who found high serum prolactin levels in 91 men with RA. A large number of their patients (n=74) used glucocorticoids and they found that higher cumulative glucocorticoid doses were significantly associated with higher prolactin levels [10]. Furthermore, in a study in 50 healthy volunteers, Dinan et al. showed that prolactin responses to buspirone were correlated with baseline cortisol levels [27]. To exclude a possible influence of glucocorticoids on prolactin levels in our study, all patients who used glucocorticoids were excluded. Thirdly, DMARDs may influence prolactin levels. To our knowledge, the effects of DMARDs on prolactin levels have not been studied. The aim of this study was to compare prolactin levels between patients with RA and healthy controls, and to investigate whether prolactin levels change after antirheumatic drug treatment. The patients were studied before and after the use of the NSAID naproxen, and after additional use of a DMARD. We studied basal serum prolactin levels and also performed insulin tolerance tests, a standardized form of stress, to assess the prolactin responses to hypoglycaemia induced stress.
SUBJECTS AND METHODS

Subjects
We included 20 patients with recent onset (< 1 year) RA who had active disease, and 20 age and sex matched healthy controls. All subjects were aged between 18 and 65 years. All patients fulfilled the revised criteria for RA of the American College of Rheumatology [28], and were consecutive patients with newly diagnosed RA attending the outpatient clinic, who were willing to participate in the study. We included only IgM rheumatoid factor positive patients, in order to avoid inclusion of other forms of arthritis than rheumatoid arthritis. Active disease was defined by a disease activity score (DAS) ≥ 3.5 [29]. Patients had not been treated with a disease modifying antirheumatic drug (DMARD) or with oral, intramuscular or intra-articular glucocorticoids. Treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) was discontinued in all patients one week prior to the study.

The patients with RA were studied at baseline, after 2 weeks and after 6 months. At baseline they were without treatment as described above. Thereafter, all patients started treatment with naproxen 500 mg twice a day and were studied again after 2 weeks. A DMARD was then added to the therapeutic regimen by the rheumatologist in charge of the patient, if indicated. The initial DMARD was sulphasalazine which could be switched to methotrexate if necessary. After 6 months of therapy the final assessments were done. Subjects could not be included if they had any condition or medication that is known to influence blood prolactin levels. Subjects using oral contraceptives were also excluded. Other exclusion criteria were anemia (Hb ≤ 6.5 mmol/l), renal or hepatic disorders, and contra-indications for undergoing the stress of an insulin tolerance test, such as cardiovascular disorders, hypertension and epilepsy. All participants voluntarily signed an informed consent form. The study protocol was approved by the hospital's ethical committee.

Methods
Disease activity
In patients with RA disease activity was assessed with a composite disease activity score (DAS), which includes the erythrocyte sedimentation rate (ESR), the Ritchie articular index, the number of swollen joints and a visual analogue scale for general well-being [29].

Prolactin levels
Serum prolactin levels were measured under basal conditions and, on separate days, during an insulin tolerance test (ITT). Blood was collected at 9:00 AM and 4:00 PM for the determination of basal prolactin levels. On the day of the ITT, subjects were fasting and placed in a supine position. At 8:30 AM a catheter was inserted in an antecubital vene and kept patent by saline solution. After a 30 minute rest, insulin (Actrapid®, Novo-Nordisk, Bagsvaerd, Denmark) was administered as a bolus injection at a dose of 0.1 units/kg body weight. Blood samples for measurement of glucose and prolactin levels were collected at 0, 20, 45, 60, 90, 120 and 180 minutes after injection. During the test, heart rate and blood pressure were measured using an automatic blood pressure recorder. The test was considered adequate if a glucose level < 2.0 mmol/l was reached. If not, the test was repeated with a 50% higher dose of insulin. This occurred in one patient, but this patient reached a glucose level < 2.0 mmol/l when the test was repeated.
All blood samples for measurement of prolactin levels were collected in dry tubes. Blood was centrifuged at 2000 g for 10 minutes and serum was stored at −20 °C until analysis. Serum prolactin levels were measured using the Spectria competitive solid-phase RIA from Farmos Diagnostica (Turku, Finland) as described earlier [30]. The sensitivity of the assay was 30 mIU/l. The within- and between-assay coefficients of variation were 5.8% and 10% at 430 mIU/l and 4.9% and 8.5% at 1200 mIU/l respectively (Normal range: 100-700 mIU/l).

**Statistical analysis**

Responses of prolactin to hypoglycemia were integrated over time as area under the response curve (AUC) from 0 to 180 minutes. The calculated AUCs are divided by 180 minutes to obtain an integrated level of prolactin during the ITT.

The primary outcome measure was the integrated serum prolactin level during the ITT. Secondary outcomes included unstimulated (basal) serum prolactin levels and levels of prolactin at individual time points during the ITT.

Comparisons between healthy controls and patients with RA were made with the unpaired t-test or with the Mann-Whitney test if data were not normally distributed. In patients with RA, the assessments after 2 weeks and after 6 months of treatment were compared with baseline with paired t-tests, or with the Wilcoxon signed rank test if data were not normally distributed. All P-values are based on 2 tailed tests and considered significant at the 0.05 level.

Spearman correlation coefficients were calculated between baseline DAS and the AUC for prolactin and between the change in DAS after 6 months and the change in AUC for prolactin after 6 months.
RESULTS

The 20 patients with active RA and the 20 healthy controls were matched for age and sex, and both groups included 3 men and 17 women. The healthy controls and the patients with RA had a mean (SD) age of 47.5 (9.8) years and 49.0 (12.0) years respectively. All 20 patients with RA were studied again after 2 weeks treatment with naproxen. Subsequently, 1 patient was treated with systemic corticosteroids and 2 patients received no DMARD therapy. These 3 patients did not participate in the assessments after 6 months. Of the 20 women in the healthy control group, 11 were pre-menopausal and of the 20 women in the RA group, 10 were premenopausal.

Disease activity

Table 1 shows the baseline disease activity and the changes from baseline after 2 weeks and after 6 months of treatment in the patients with recent onset RA. After 2 weeks use of naproxen the DAS decreased significantly with a mean value of 0.7 (p=0.0003). The individual components of the DAS also showed significant decreases with the exception of the ESR, which remained unchanged. After 6 months of additional treatment with either sulphasalazine or methotrexate, the DAS showed further improvement (mean decrease 1.6 units, p=0.0002 versus baseline). The ESR however, again remained unchanged when compared to baseline values.

Table 1. Disease activity: baseline values and changes from baseline after 2 weeks and 6 months of antirheumatic drug therapy

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=20)</th>
<th>Change from baseline</th>
<th></th>
<th>Change from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean  SD</td>
<td>mean  SD *</td>
<td></td>
<td>mean  SD *</td>
</tr>
<tr>
<td>DAS (units)</td>
<td>4.3  0.7</td>
<td>-0.7  0.7</td>
<td>0.0003</td>
<td>-1.6  1.2</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>21.6  24.7</td>
<td>3.4  8.1</td>
<td>0.13</td>
<td>2.6  32.2</td>
</tr>
<tr>
<td>RAI (units)</td>
<td>16.7  7.8</td>
<td>-4.3  4.9</td>
<td>0.001</td>
<td>-10.1  6.5</td>
</tr>
<tr>
<td>Number swollen joints</td>
<td>11.4  6.3</td>
<td>-2.4  5.1</td>
<td>0.02</td>
<td>-4.6  8.1</td>
</tr>
<tr>
<td>VAS general well-being (mm)</td>
<td>52.3  1.9</td>
<td>-12.8  19.1</td>
<td>0.007</td>
<td>-25.1  25.9</td>
</tr>
</tbody>
</table>

* for comparison with baseline

DAS Disease activity score
ESR Erythrocyte sedimentation rate
RAI Ritchie articular index
VAS Visual analogue scale (0 – 100 mm)
Prolactin levels

Table 2 summarizes the prolactin levels at baseline and the changes therein after 2 weeks and 6 months of antirheumatic treatment. At baseline the unstimulated prolactin levels were not different between the RA patients and the healthy controls.

**Table 2.** Basal serum levels of prolactin and integrated AUC of serum prolactin levels during ITT in healthy controls, and in patients with recent onset RA: baseline and changes from baseline after 2 weeks and 6 months of antirheumatic treatment.

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=20)</th>
<th>Rheumatoid arthritis (n=20)</th>
<th>Change from baseline in rheumatoid arthritis</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
<td>SD</td>
<td>P*</td>
<td>mean</td>
</tr>
<tr>
<td>Basal prolactin levels (mIU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:00 AM</td>
<td>317</td>
<td>169</td>
<td>276</td>
<td>159</td>
<td>&gt;0.2</td>
<td>11</td>
</tr>
<tr>
<td>4.00 PM</td>
<td>235</td>
<td>129</td>
<td>246</td>
<td>148</td>
<td>&gt;0.2</td>
<td>-12</td>
</tr>
<tr>
<td>Prolactin during ITT (mIU/L):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC/180 min ***</td>
<td>552</td>
<td>287</td>
<td>409</td>
<td>313</td>
<td>0.03</td>
<td>-6</td>
</tr>
</tbody>
</table>

* for the comparison between healthy controls and rheumatoid arthritis patients
** for the comparison with baseline in rheumatoid arthritis patients
*** AUC: area under the curve

There were no significant differences between the groups in the hypoglycemia nadir that was reached during the ITT. The serum prolactin levels during the ITT however, were significantly lower in RA patients than in healthy controls between 30 and 90 minutes after the bolus injection with insulin (figure 1). This resulted also in a significantly lower AUC for prolactin during the ITT in RA patients. After 2 weeks treatment with naproxen, no significant changes occurred in either basal or stimulated serum prolactin levels. After 6 months of antirheumatic drug treatment serum prolactin levels during the ITT were significantly higher than at baseline between 60 and 120 minutes after the bolus injection with insulin. The AUC for prolactin was also significantly higher at 6 months compared to baseline.

At baseline, the DAS correlated negatively with the AUC for prolactin (r = -0.58; p=0.008) in patient with RA. After 6 months of treatment, the improvement in DAS correlated with the change in AUC for prolactin (r=0.48; p=0.05).
DISCUSSION

In our study, untreated newly diagnosed patients with active RA have basal serum prolactin levels that are not different from healthy controls, but their prolactin responses to the stress induced by hypoglycaemia are decreased compared with healthy controls. Ideally, we should have measured the pre-menopausal women at the same phase of the menstrual cycle at each time-point of the study, but for practical reasons, this was not possible. Because of the large number of patients and the similar number of pre-menopausal and post-menopausal subjects in each group, we expected that this would not influence our results. The fact that we did not find any difference in basal prolactin levels between the groups, in our view confirms that assumption.

Previous studies of prolactin levels in RA patients have shown contradictory results. As discussed before, several explanations may be offered to account for the different results in the literature. The possibility that NSAIDs influence prolactin secretion, to us now seems unlikely, as our longitudinal results indicate that 2 weeks of treatment with the NSAID naproxen has no effect on either basal or stimulated prolactin levels. However, we are aware that this does not exclude a possible effect of other NSAIDs. The possibility of an influence of glucocorticoids on prolactin levels was excluded in this study as patients using glucocorticoids were excluded. The effects of DMARDs on prolactin levels have not been studied before. In our study prolactin responses to hypoglycaemia induced stress increase significantly after 6 months of treatment with either sulphasalazine or methotrexate to levels comparable with the healthy controls. We hypothesize that this increase in prolactin response after 6 months is related to the considerable decrease in disease activity observed at that time point. The significant negative correlation between disease activity and prolactin levels that we found at baseline and the significant positive correlation between the improvement of the DAS and the increase in prolactin levels after 6 months of treatment, support this view. Göbjörnssen et al. [14], in a study in 18 patients with active untreated RA, found a decreased prolactin response to MRH (multiple releasing hormone) test, but a normalisation of the prolactin response after treatment with corticosteroid therapy, which supports an impaired prolactin response due to inflammatory stimuli, consistent with our hypothesis. In a recent study by Pool et al. [32], basal prolactin levels in patients with RA and SLE did not differ from healthy volunteers, but in response to exercise-induced stress, both groups showed only small and insignificant increases in prolactin levels, in contrast to healthy volunteers, who showed a large increase, comparable to our findings in response to ITT-induced stress.

The exact mechanism by which the prolactin response to stress is decreased in active RA, remains unclear. One could speculate, that elevated levels of pro-inflammatory cytokines like IL-6 and IL-1β, which are found during active disease in RA, may have an inhibitory effect on prolactin secretion. This is supported by a study in rats, that showed that interleukin-1β was able to inhibit prolactin release [33]. However, in the litterature, inhibitory as well as stimulatory effects of cytokines on prolactin release have been described, and future research will be needed to clarify this issue.

Finally, another possible explanation for the differences in the literature could be the use of different prolactin stimulation tests. Chikanza et al. studied prolactin responses after major surgery, a non-standardized stimulus for prolactin release [5]. The increased prolactin responses they found could not be reproduced in a similar study [19]. Other investigators have employed thyrotropin releasing hormone (TRH) tests [8,9,18,20]. Templ et al.[18] and Gutiérrez et al.[20] found normal prolactin responses to TRH, while Jorgensen et al.
found increased responses [8,9]. ITTs have not been used before to assess prolactin responses in RA patients. Normal or even increased prolactin responses to TRH may suggest that the decreased prolactin responses to hypoglycaemia that we observed in untreated patients with RA, may be due to hypothalamic rather than pituitary defects in RA patients. This hypothesis should be studied in more detail in future investigations.

To our knowledge, this is the first longitudinal study of prolactin levels in patients with newly diagnosed RA. We conclude that prolactin responses to hypoglycaemia are decreased in patients with active RA. The disturbance may be a consequence of active disease because prolactin responses increase towards normal values when disease activity is reduced after 6 months of antirheumatic treatment.

Our data contradict the hypothesis that increased prolactin levels play a role in the pathogenesis of RA. Instead, we found decreased prolactin responses to stress in active RA, which may be considered as part of the normal anti-inflammatory defense mechanism.
ACKNOWLEDGMENTS
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REFERENCES


Figure 1. Serum prolactin levels during ITT of healthy controls, and of patients with RA at baseline, after 2 weeks of treatment with naproxen, and after 6 months of additional treatment with a DMARD. * = Significant difference between HC and RA patients at baseline: p < 0.05.