Possible role of leptin in hypoandrogenicity in patients with systemic lupus erythematosus and rheumatoid arthritis

P Härle, G Pongratz, C Weidler, R Büttner, J Schölmerich, R H Straub

**Background:** Hypoandrogenicity is common in obesity and in chronic inflammatory diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Adrenal androgens such as androstenedione (ASD) and dehydroepiandrosterone (DHEA) sulphate are low, which partly depends on the influence of TNF in chronic inflammatory diseases. Leptin is stimulated by TNF and is associated with hypoandrogenicity in non-inflammatory conditions.

**Objective:** To study the interrelation between serum levels of leptin and adrenal steroids in SLE and RA.

**Methods:** In a retrospective study, serum levels of leptin, ASD, DHEA, and 17-hydroxyprogesterone (17OHP) were measured by ELISA, and serum levels of cortisol by radioimmunoassay in 30 patients with RA, 32 with SLE, and 54 healthy control subjects (HS).

**Results:** In SLE and RA, serum levels of ASD correlated negatively with serum levels of leptin (p < 0.01) independently of prior prednisolone treatment in patients with SLE (p = 0.013) and tended to be independent of prednisolone in patients with RA (p = 0.067). In a partial correlation analysis, this interrelation remained significant after controlling for daily prednisolone dose in both patient groups. In both patient groups, serum leptin levels correlated negatively with the molar ratio of serum ASD/serum cortisol and serum ASD/serum 17OHP, and positively with the molar ratio of serum DHEA/serum ASD.

**Conclusions:** The negative correlation of serum leptin and ASD or, particularly, ASD/17OHP, together with its known anti-androgenic effects indicate that leptin is also involved in hypoandrogenicity in patients with SLE and RA. Leptin may be an important link between chronic inflammation and the hypoandrogenic state.
according to the criteria of the American College of Rheumatology. In the latter patients, clinical activity was assessed by the SLE Disease Activity Index (SLEDAI). Table 1 shows the basic characteristics of both disease groups and their treatment. Clearly, patients in both disease groups presented mild to moderate disease activity. Patients of both disease groups entered the study consecutively between 2000 and 2002. Some of the patients with RA and SLE were the subject of a recent study which dealt with other aspects of these diseases (manuscript submitted).

For comparison, 54 white healthy control subjects (HS) were included (mean (SEM) age 40.8 (1.5) years), and their health status was verified by a 33 item questionnaire, as previously described. Fertile women (HS and patients) were not taking contraceptives and they were in the early to mid-follicular phase of the menstrual cycle as estimated by serum progesterone levels (table 1). Information about the HS was collected between 2000 and 2002 as part of another investigation which dealt with different aspects (see above, manuscript submitted).

Because this was a retrospective study, we did not measure body mass and body height so that comparisons of serum leptin levels between groups were not made. Our intention was to focus only on relations between serum levels of leptin and adrenal steroids within the respective groups. Because we would expect raised leptin levels in subjects with increased body mass index, the mentioned significant correlations may be explained by the subjects’ body mass index. We believe that the missing body mass index did not markedly influence our report.

On the day of the visit, between 8 00 and 10 00 am, blood for further determination of leptin and steroid hormones was taken, and serum was stored at \(-20^\circ C\) in adequate aliquots.

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**Figure 1** Pathways of steroidogenesis. A line with a bar at the end demonstrates that the respective mediator inhibits the enzyme step (tumour necrosis factor (TNF)).

Abbreviations: 3βHSD, 3β-hydroxysteroid dehydrogenase; 11βHSD I and II; 11β-hydroxysteroid dehydrogenase types I and II; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulphate; DST, DHEA sulphotransferase; IL6, interleukin 6; P450c11, 11β-hydroxylase; P450c17, 17α-hydroxylase and 17/20-lyase (double enzyme step); P450c21, 21α-hydroxylase; P450scc, side chain cleavage enzyme; ST, DHEAS sulphatase; STAR, steroidogenic acute regulatory protein; TNF, tumour necrosis factor.

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investigation of their blood samples. Patients and HS lived in the catchment area of the University Hospital Regensburg, Bavaria.

**Laboratory parameters**

Radioimmunometric assay was used for the quantitative determination of serum levels of cortisol (Coulter Immunotech, Marseilles, France; detection limit 10 nmol/l; cross reactivity with prednisolone ≤6%). Serum levels of ASD (IBL, Hamburg, Germany; detection limit 0.15 nmol/l), 17-hydroxyprogesterone (17OHP; IBL, Hamburg, Germany; detection limit 0.3 nmol/l), and DHEA (Diagnostic Systems Laboratory, Webster, Texas; detection limit 0.13 nmol/l) were measured by immunometric enzyme immunoasays. Serum leptin was measured by an enzyme linked immunosorbent assay (ELISA) with a detection limit of 7.8 pg/ml (R&D Systems, Wiesbaden, Germany). As indicated by the suppliers, intra-assay and interassay coefficients of variation for all assays were below 10%.

To demonstrate a preponderance of one serum hormone over another, the molar ratio of these hormones was calculated (given without unit). This procedure detects a hormonal shift through one to more adrenalone steps which can demonstrate a preponderance of an adrenal pathway (fig 1): ASD/cortisol for P450c21, P450c11, and 17/20-lyase (2nd reaction of the P450c17) into the direction of DHEA and ASD, ASD/17OHP for the 17/20-lyase (2nd reaction of the P450c17) into the direction of ASD, and DHEA/ASD for the 3β-hydroxysteroid dehydrogenase into the direction of DHEA (fig 1).

**Statistical analysis**

Investigation of an interrelation between two variables was done using Spearman rank correlation analysis (SPSS/PC for Windows, version10.0.5, SPSS, Inc, Chicago). Medians within groups were compared by Mann-Whitney signed rank test (SPSS). To control an interrelation between two variables for a third control parameter partial correlation analysis was used (SPSS). Values of \( p<0.05 \) were considered to be significant and results are given as mean (SEM).

**RESULTS**

In patients with SLE and RA, serum levels of ASD correlated negatively with serum leptin concentrations (fig 2) which was similar in the subgroups with and without prior prednisolone treatment (fig 2). In all patients with SLE and RA including those with prior prednisolone treatment, a highly significant interrelation existed (SLE: \( R_{\text{rank}} = -0.618, p<0.0001 \), and RA: \( R_{\text{rank}} = -0.601, p<0.0001 \), respectively). After controlling this interrelation for daily prednisolone dose this particular negative correlation remained significant (SLE: \( R_{\text{partial}} = -0.483, p = 0.006 \), and RA: \( R_{\text{partial}} = -0.439, p = 0.028 \), respectively). No such correlations were seen in HS (fig 2, lower panel).

To estimate the hormonal preponderance of ASD versus cortisol, the molar ratio of these two hormones was calculated. This ratio correlated negatively with serum leptin levels in all patients, including those with prior prednisolone treatment (fig 3). In patients with SLE, this interrelation remained significant after controlling for daily prednisolone dose (SLE: \( R_{\text{partial}} = -0.556, p = 0.001 \). In contrast, in patients with RA controlling for prednisolone intake abolished the negative correlation between serum leptin and this particular ratio (RA: \( R_{\text{partial}} = -0.313, p = 0.210 \). In HS, no such negative correlation was seen (fig 3, lower panel).

To directly investigate the shift from 17-OHP to ASD, the molar ratio of these hormones was calculated (fig 1). In support of above mentioned results, this ratio also correlated negatively with serum leptin levels (fig 4). In addition, both in patients with SLE and RA this negative correlation remained significant after controlling for daily prednisolone dose (SLE: \( R_{\text{partial}} = -0.400, p = 0.026 \); RA: \( R_{\text{partial}} = -0.448, p = 0.022 \). These correlations were not significantly negative in HS (fig 4, lower panel).

The data clearly indicate a reduction of the adrenal androgen ASD in relation to upstream steroid hormones. To estimate the role of the 3β-hydroxysteroid dehydrogenase (fig 1), the molar ratio of DHEA/ASD was calculated (fig 1). Interestingly, this ratio correlated positively with serum leptin levels in all patients with SLE and RA (fig 5). This positive interrelation remained significant in those patients with SLE and RA without prior prednisolone treatment (SLE: **Table 1** Basic characteristics of healthy subjects and patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). The patients should not be compared owing to a different mean age of the groups.

<table>
<thead>
<tr>
<th></th>
<th>SLE</th>
<th>RA</th>
<th>Healthy subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>32</td>
<td>30</td>
<td>54</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.1 (2.1)</td>
<td>56.1 (2.4)</td>
<td>40.8 (1.5)</td>
</tr>
<tr>
<td>Sex (F/M, No (%))</td>
<td>24/8 (75/25)</td>
<td>22/8 (73/27)</td>
<td>26/28 (48/52)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>8.0 (1.5)</td>
<td>10.8 (1.9)</td>
<td>NA</td>
</tr>
<tr>
<td>SLEDAI (points)</td>
<td>10.1 (1.5)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Number of swollen joints</td>
<td>NA</td>
<td>4.1 (1.5)</td>
<td>NA</td>
</tr>
<tr>
<td>Number of painful joints</td>
<td>NA</td>
<td>6.6 (1.6)</td>
<td>NA</td>
</tr>
<tr>
<td>Serum progesterone (nmol/l)*</td>
<td>2.6 (0.4) [0.0–6.32]</td>
<td>1.5 [0.3] [0.1–5.0]</td>
<td>3.0 (0.4) [0.2–6.3]</td>
</tr>
<tr>
<td>ESR (mm/1st h)</td>
<td>25.0 (3.3)</td>
<td>27.7 (3.9)</td>
<td>NA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Prednisolone, No (%)</td>
<td>22 (69)</td>
<td>20 (67)</td>
<td>NA</td>
</tr>
<tr>
<td>Prednisolone (mg/day)</td>
<td>9.4 (3.4)</td>
<td>6.5 (1.8)</td>
<td>NA</td>
</tr>
<tr>
<td>Methotrexate, No (%)</td>
<td>2 (6)</td>
<td>9 (30)</td>
<td>NA</td>
</tr>
<tr>
<td>NSAIDs, No (%)</td>
<td>13 (41)</td>
<td>11 (37)</td>
<td>NA</td>
</tr>
<tr>
<td>Leflunomide, No (%)</td>
<td>0 (0)</td>
<td>8 (27)</td>
<td>NA</td>
</tr>
<tr>
<td>Anti-TNF strategy, No (%)</td>
<td>NA</td>
<td>6 (20)</td>
<td>NA</td>
</tr>
<tr>
<td>Sulfasalazine, No (%)</td>
<td>0 (0)</td>
<td>2 (7)</td>
<td>NA</td>
</tr>
<tr>
<td>Cyclophosphamide, No (%)</td>
<td>2 (6)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Azathioprine, No (%)</td>
<td>12 (38)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data are given as means (SEM), No (%), and ranges in brackets. 
*Mean serum level of progesterone in women (normal range in the follicular phase <6.5 nmol/l); †anti-TNF strategies were either infliximab or etanercept. 
ESR, erythrocyte sedimentation rate; NA, not applicable; NM, not measured; NSAIDs, non-steroidal anti-inflammatory drugs; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index.

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Rank = 0.648, p = 0.043; RA: Rank = 0.833, p = 0.005). There was no such correlation in HS (fig 5, lower panel).

Because leptin serum levels were described as higher in women than in men, we compared our readout parameters between women and men within the respective disease groups. Serum leptin levels were higher in women than in men, and this difference was significant in HS and patients with RA (table 2). No differences were found for serum levels of ASD (table 2) and all calculated molar ratios (not shown).

DISCUSSION
This study demonstrates an inverse interrelation between serum levels of leptin and ASD or molar ratios of serum levels of ASD/cortisol or ASD/17OHP. This was accompanied by a positive correlation between serum leptin and the molar ratio of serum DHEA/serum ASD (fig 1). All these correlations were particularly stable in patients with SLE irrespective of prior prednisolone treatment.

Several recent studies demonstrated the inhibitory influence of leptin on gonadal and adrenocortical ASD production, which is achieved by direct inhibition of the respective P450 enzyme mRNA expression in steroid hormone producing cells. Our studies in chronic inflammatory diseases corroborate the possibility that leptin may have an inhibitory influence on ASD secretion which was not seen in our HS. In view of the known in vitro data of leptin induced inhibition of ASD (reviewed by Glasow and Bornstein), our study supports the concept that the important enzyme step of the 17/20-lyase (2nd reaction of the P450c17) is particularly altered in these patients (fig 1). Moreover, owing to the positive correlation between serum leptin and the molar ratio serum DHEA/serum ASD, 3β-hydroxysteroid dehydrogenase may also be altered by leptin (not in HS). However, this latter fact has not been investigated in vitro in adrenocortical cells and, thus, remains to be elucidated. Because we and others believe that androgens are anti-inflammatory in chronic inflammatory diseases, their continuous reduction is unfavourable for the disease outcome. Loss of ASD would lead to a loss of testosterone and dihydrotestosterone (fig 1). The question arises as to the more general role of leptin during an inflammatory episode and why leptin should have a proinflammatory role.

Leptin from adipose tissue is stimulated by proinflammatory cytokines such as TNF and interleukin 1β which has been demonstrated several times. These cytokines seem to stimulate short term release of stored leptin, although its

Figure 2. Interrelation between serum levels of ASD and leptin in patients with SLE, RA, and HS. Open symbols (broken line) stand for patients with prior prednisolone treatment whereas black symbols (solid line) represent patients without prior prednisolone treatment. The linear regression lines, the Spearman rank correlation coefficients (R_{rank}) and the p values are given in the panels. Direct comparison of patients with SLE, RA, and HS is not meaningful because of the unknown body mass index (take notice of the different scales).
production may even be inhibited during long term in vitro stimulation with TNF. Thus, the acute cytokine driven rise in leptin may support the initial proinflammatory response. Indeed, human leptin stimulates proliferation and activation of human circulating monocytes through the leptin receptor, which is a member of the interleukin 6 receptor related cytokine receptors. Leptin is a fundamental factor for human T cell proliferation and it induces T helper type 1 immune reactions. Moreover, fat mass in humans is directly related to white blood count, which is closely related to leptin serum levels, and leptin stimulates oxidative species production by stimulated polymorphonuclear neutrophils. Thus, leptin may be an “acute phase protein of fat tissue” which supports the immune system during a short term infectious disease. Indeed, leptin deficient mice exhibit impaired host defence in Gram negative pneumonia, and starvation with low serum leptin levels leads to immunosuppression. Interestingly, serum leptin levels are increased in survivors of sepsis as compared with non-survivors. All these factors indicate that leptin has been evolutionarily conserved for an acute inflammatory response. During some chronic inflammatory disease, serum levels of leptin are also increased. In our study we could not confirm this fact because we did not match for body mass index and age (thus, comparison of the groups is not allowed). As in the acute infectious condition, most probably the proinflammatory load increases serum leptin which depends on fat mass. One may speculate that leptin is a systemic indicator of the available energy resources: it may be necessary for the fine tuning of the vastly energy consuming immune response. In this respect, it supports the proinflammatory pathways of the immune system.

In view of these general immune supportive aspects of leptin, leptin is probably a proinflammatory mediator which was evolutionarily saved for the acute inflammatory episode. In this respect, inhibition of leptin induced androgens is an indication of its proinflammatory role. TNF, like leptin, directly inhibits adrenal steroids by modulating P450c17 and others. In a recent long term study in patients with RA, anti-TNF treatment obviously favoured androgen secretion, which demonstrates the detrimental influence of this cytokine on androgens. Because both TNF and leptin can inhibit androgen secretion in parallel, one may speculate that the negative correlations between serum leptin and ASD (and ratios) are only to be found in patients with a high proinflammatory load and, thus, did not occur in our HS.

Figure 3 Interrelation between the molar ratio of serum ASD/serum cortisol and leptin in all patients with SLE, RA, and HS. In both disease groups, patients with and without prior prednisolone treatment were included. The linear regression line, the Spearman rank correlation coefficient and the p value are given in the panels. Direct comparison of patients with SLE, RA, and HS is not meaningful because of the unknown body mass index (take notice of different scales).
In conclusion, in patients with SLE and RA our study supports the known concept that leptin inhibits androstenedione secretion. In chronic inflammatory diseases, this phenomenon may add to the well known hypoandrogenicity. Because leptin is a proinflammatory mediator and androgens are anti-inflammatory, preponderance of leptin and hypoandrogenicity may help to perpetuate chronic inflammatory diseases.

**Figure 4** Intercalation between the molar ratio of serum ASD/serum 17OHP and leptin in all patients with SLE, RA, and HS. In both disease groups, patients with and without prior prednisolone treatment were included. The linear regression line, the Spearman rank correlation coefficient (R_{Spear}) and the p value are given in the panels. Direct comparison of patients with SLE, RA, and HS is not meaningful because of the unknown body mass index (take notice of the different scales).

In conclusion, in patients with SLE and RA our study supports the known concept that leptin inhibits androstenedione secretion. In chronic inflammatory diseases, this phenomenon may add to the well known hypoandrogenicity.

**Table 2** Basic characteristics of healthy subjects and patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA)

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum leptin (pg/ml)</td>
<td>14490 (2946)</td>
<td>4715 (610)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum ASD (nmol/l)</td>
<td>13.5 (1.3)</td>
<td>15.6 (1.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum leptin (pg/ml)</td>
<td>11999 (1643)</td>
<td>6319 (2167)</td>
<td>0.064</td>
</tr>
<tr>
<td>Serum ASD (nmol/l)</td>
<td>4.6 (0.9)</td>
<td>6.7 (2.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum leptin (pg/ml)</td>
<td>3973 (572)</td>
<td>2186 (554)</td>
<td>0.047</td>
</tr>
<tr>
<td>Serum ASD (nmol/l)</td>
<td>1.9 (0.3)</td>
<td>3.0 (0.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are given as means (SEM).
ASD, androstenedione.
The values are not given for comparison of subgroups because body mass index was not available. For this particular leptin assay, the supplier provided mean values for women and men of 20.676 pg/ml (range 3877–77,273) and 47.60 pg/ml (2203–11,149), respectively (R&D Systems, Wiesbaden, Germany).
Figure 5 Interrelation between the molar ratio of serum (DHEA)/serum androstenedione and serum leptin in all patients with SLE, RA, and HS. In both disease groups, patients with and without prior prednisolone treatment were included. The linear regression line, the Spearman rank correlation coefficient ($R_{\text{rank}}$), and the p value are given in the panels. Direct comparison of patients with SLE, RA, and HS is not meaningful because of the unknown body mass index (take notice of different scales).

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REFERENCES

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