Anti-TNF antibody therapy improves glucocorticoid-induced insulin-like growth factor-1 (IGF-1) resistance without influencing myoglobin and IGF-1 binding proteins 1 and 3

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Keywords: Rheumatoid arthritis, adalimumab, insulin-like growth factor 1, IGF-1 binding protein-1, IGF-1 binding protein-3

Running title: Anti-TNF and IGF-1 in RA
Objective: Insulin-like growth factor-1 (IGF-1) is an important determinant of muscle mass because it promotes growth and suppresses protein degradation. IGF-1 levels are decreased in patients with rheumatoid arthritis (RA) and juvenile idiopathic arthritis due to inflammation-induced inhibition of IGF-1 synthesis. In parallel, glucocorticoids induce IGF-1 resistance and add to muscle degradation. This study aimed to investigate the influence of anti-TNF antibody therapy (anti-TNF) with adalimumab on levels of myoglobin (degradation marker) and IGF-1 in RA patients with and without prednisolone therapy.

Methods: In this longitudinal study with subcutaneous adalimumab, 32 long-standing RA patients were included (16 with and 16 without prednisolone). We measured IGF-1, IGF-1 binding protein-1 (IGFBP-1), IGFBP-3, and myoglobin by ELISA techniques.

Results: RA patients demonstrated normal myoglobin serum levels. However, patients with prednisolone had elevated levels of myoglobin as compared to patients without prednisolone indicating increased muscle degradation. Upon treatment with anti-TNF, myoglobin levels did not change in both patient groups. Serum levels of IGF-1 were increased in patients with versus without prednisolone indicating IGF-1 resistance (221 ± 23 vs. 122 ± 14 µg/l, p<0.001). Adalimumab therapy decreased elevated IGF-1 levels in patients with prednisolone, which after 12 weeks of treatment reached the level of patients without prednisolone. Serum levels of IGFBP-1 and IGFBP-3 were not different in the two groups and anti-TNF did not change these concentrations.

Conclusions: Anti-TNF antibody therapy over 12 weeks seems to improve glucocorticoid-induced IGF-1 resistance without influencing myoglobin and IGF-1 binding proteins. Thus, in RA patients on glucocorticoids with generally decreased muscle mass anti-TNF therapy with adalimumab exerts favourable effects.
A majority of patients with rheumatoid arthritis (RA) suffer from decreased muscle function and loss of body cell mass by presently unknown mechanisms. High levels of TNF and parallel glucocorticoid therapy were thought to be important elements for these alterations in RA patients. One key element in maintaining muscle mass is insulin-like growth factor-1 (IGF-1) by promoting muscle growth and suppressing muscle degradation. IGF-1 has additional favourable effects on TNF-induced cartilage degradation. Furthermore, TNF, one major proinflammatory cytokine in RA, inhibits synthesis of IGF-1 from liver cells, and it also inhibits IGF-1 mediated anabolic effects on peripheral tissue. From these data, one would expect an increase of IGF-1 serum levels during anti-TNF therapy in patients with RA, which has never been investigated.

In addition, parallel glucocorticoid therapy may influence the effects of anti-TNF therapy because glucocorticoids per se can lead to IGF-1 resistance. IGF-1 resistance has been demonstrated during glucocorticoid therapy by displaying an increase of IGF-1 serum levels. A glucocorticoid-related increase of IGF-1 in the presence of increased muscle degradation is a marker of muscular IGF-1 resistance. A very similar phenomenon exists with respect to insulin resistance: Glucocorticoids increase insulin resistance. Furthermore, patients with rheumatoid arthritis demonstrate insulin resistance, which is improved during anti-TNF therapy.

Since serum levels of IGF-1 and its most important binding proteins, IGF-1 binding protein 1 (IGFBP-1) and IGFBP-3, have never been studied during anti-TNF therapy in RA patients, this study aimed to shed light on these parameters during adalimumab therapy over 12 weeks. In addition, we focussed on IGF-1 resistance under glucocorticoid therapy with prednisolone.

PATIENTS AND METHODS

Patients, adalimumab therapy and blood samples
In this study with the human monoclonal antibody adalimumab (Abbott S.p.A., Campoverde di Aprilia, Italy), we included 32 Caucasian RA patients with RA fulfilling the ACR criteria for RA. The patients were selected according to the inclusion criteria of the Adalimumab Research in Active RA study (ReAct). A total of 16 patients received parallel prednisolone whereas another 16 patients did not receive parallel or prior (6 months before) prednisolone therapy. Most patients were administered additional methotrexate (stable throughout this study) but no other immunosuppressive drugs. Baseline characteristics of patients are given in table 1.

Patients were assigned to receive single self-injections of adalimumab subcutaneously at 40 mg every other week. Efficacy assessments included ACR and EULAR response criteria. A baseline blood sample was taken 1 to 2 weeks before the start of adalimumab therapy. Anti-TNF antibodies were infused on week 0, 2, 4, 6, 8, 10, and 12. For this study, patients were clinically investigated and blood was drawn between 08:00 and 09:00 in the morning when the patients visited the outpatient clinic on the baseline day, week 2, 6, and 12. The blood was immediately centrifuged and serum was stored on – 80°C. The Ethics Committee of L. Sacco University Hospital, Italy approved the study.

Laboratory parameters
We used enzyme immunoassays for the quantitative determination of serum levels of IL-6 (high sensitivity Quantikine, R&D Systems, Minneapolis, MN, USA), myoglobin (Life Diagnostics, Inc., West Chester, PA; normal range according to the manufacturer 12 to 90 ng/ml), IGF-1 (IDS, Bolden, England; normal range according to the manufacturer of subjects aged 60 yr: 30 to 200 μg/l), IGFBP-1 (Oy Medix Biochemica, Kauniainen, Finland), and IGFBP-3 (Biosource Europe, Nivelles, Belgium). Intraassay and interassay coefficients of variation for all tests were below 10%.

Statistical Analysis
Medians between different groups were compared by the non-parametric Mann-Whitney test (SPSS/PC, Advanced Statistics, V11.5.1, SPSS Inc., Chicago ). A decrease or increase of a variable over time (during anti-TNF therapy) was tested by means of the non-parametrical Friedman test (SPSS). An interrelation between two parameters was tested by the non-parametrical Spearman rank correlation analysis (SPSS). p < 0.05 was the significance level.

RESULTS

Antinflammatory effects of adalimumab therapy
Adalimumab therapy exerted excellent antinflammatory effects in patients with RA with or without glucocorticoids as investigated by the number of swollen joints, the number of tender joints, patients’ global assessment
of pain, and serum levels of IL-6 (Table 1). It seems that these effects were more marked in patients without glucocorticoid therapy (Table 1).

Influence of glucocorticoid therapy on muscle degradation and effects of adalimumab
Baseline serum myoglobin levels were significantly higher in patients on prednisolone therapy as compared to patients without glucocorticoids (Fig. 1). This indicates a higher degree of muscle degradation in patients on glucocorticoids. After controlling for age, this difference remained statistically significant at week 2 and 12 (not at baseline and week 6). During the course of anti-TNF therapy, myoglobin levels remained stable in patients with and without prednisolone therapy (Fig. 1). Anti-TNF did not change myoglobin levels, which may indicate that TNF is not the main player for this glucocorticoid-induced phenomenon.

Influence of glucocorticoid therapy on IGF-1 serum levels and effects of adalimumab
Patients receiving prednisolone demonstrated markedly higher levels of IGF-1 as compared to patients without glucocorticoid therapy (Fig. 2A), although these patients were somewhat older and lower IGF-1 serum levels are expected (Table 1). This was particularly evident at baseline where IGF-1 serum levels of more than 50% of patients with prednisolone exceeded the normal age-related range given by the manufacturer (30 to 200 µg/l). In the presence of increased muscle degradation (myoglobin!), this phenomenon is called IGF-1 resistance.

During the course of anti-TNF therapy, serum levels of IGF-1 did not markedly change in patients without prednisolone therapy (Fig. 2B). However, in patients with prednisolone therapy elevated IGF-1 serum levels decreased on average by more than 50 µg/l (Fig. 2B). The decrease was statistically significant as objectified by the Friedman test (Fig. 2B). At 12 weeks of adalimumab therapy, IGF-1 serum levels were not different in the two groups (Fig. 2B), which indicate a normalisation of IGF-1 resistance.

Influence of glucocorticoid therapy on IGF-1 binding proteins and effects of adalimumab
At baseline, the two major binding proteins of IGF-1, IGFBP-1 and IGFBP-3, were not different in patients with and without glucocorticoid therapy (data not shown). This remained stable throughout adalimumab therapy (data not shown). Furthermore, adalimumab therapy did not markedly alter the course of serum levels of these binding proteins (data not shown).

Influence of IL-6 and medication on IGF-1 and its binding proteins
There was no interrelation between serum levels of IGF-1 and serum IL-6 in patients with and without prednisolone (data not shown). Neither existed an interrelation with C-reactive protein or erythrocyte sedimentation rate and IGF-1 serum levels (data not shown).

In patients with prednisolone, serum IL-6 correlated positively with serum IGFBP-1 at week 2 (R_{Rank} = 0.500, p=0.048), at week 6 (R_{Rank} = 0.598, p=0.015), and at week 12 (R_{Rank} = 0.797, p<0.001). The positive interrelation increased during adalimumab therapy at generally much lower levels of IL-6 (compare table 2). However, no such interrelation existed in patients without prednisolone (data not shown). IL-6 did not correlate with IGFBP-3 in both patient groups.

Furthermore, no interrelation was detected between IGF-1, IGFBP-1, or IGFBP-3 and intake of NSAIDs, or chloroquine/hydroxychloroquine (data not shown).

DISCUSSION
The hypothalamus – pituitary – liver – muscle (HPLM) axis is a delicate reflex loop stabilising muscle mass (Fig. 3). The liver, upon stimulation with growth hormone, produces IGF-1, IGF-1 stimulates muscle growth, and IGF-1 itself inhibits its own secretion from the liver (Fig. 3). IGF-1 – induced inhibition of liver IGF-1 is probably dependent on consumption of liver IGF-1 within the muscle (binding to receptors and local degradation). Both, TNF and exogenous glucocorticoids inhibit muscle growth by stimulating protein degradation pathways in the muscle and by interfering with IGF-1 signalling (Fig. 3). Glucocorticoids exert an additional inhibiting effect on muscle by inducing the muscle growth inhibiting factor myostatin. One study in burn-induced muscle degradation showed that exogenous and endogenous glucocorticoids induce myostatin. Furthermore, it was shown that glucocorticoids inhibit production of muscular IGF-1 leading to a decrease of negative feedback regulation on liver IGF-1 (Fig. 3). The decrease of negative feedback would increase serum levels of IGF-1 (Fig. 3)

In addition, TNF inhibits growth hormone-induced IGF-1 production from the liver. We have not tested serum levels of growth hormone, a decrease of which might be causal for low serum levels of IGF-1 (as observed). Such a decrease of growth hormone would be an unwanted effect of high TNF levels, which can add to the negative sequelae in RA such as atherosclerosis and elevation of lipid levels. However, since growth hormone kinetics are not notably changed in RA patients in general, the effect is likely dependent on another mechanism than growth hormone secretion and action in the liver. In addition, since exogenous glucocorticoids inhibit pituitary growth hormone release, one would have expected low levels of growth hormone-stimulated
IGF-1 in patients with prednisolone treatment. However, exactly the opposite was observed, which is in disagreement with a marked influence of growth hormone on the observed phenomena.

In RA patients, TNF plays a dominant proinflammatory role and these patients are often additionally treated with exogenous glucocorticoids (as in the ReAct study). Thus, in RA patients two important muscle growth-inhibiting factors are present, which may largely influence muscle homeostasis (Fig. 3). This is an important factor for disability in these patients, who additionally suffer from rheumatoid cachexia. This present study demonstrates that neutralising TNF by anti-TNF therapy decreases IGF-1 serum levels in patients with parallel glucocorticoid therapy (in the presence of somewhat elevated myoglobin levels). It might also be that our patients on glucocorticoids were somewhat more ill than those without, which may have increased their myoglobin levels. This is, however, not supported by the baseline characteristics given in table 1. Interestingly, myoglobin did no change during anti-TNF therapy but this may depend on other proinflammatory factors and long-standing effects on muscle degradation. Removal of the important proinflammatory TNF will probably attenuate muscle wasting by increasing IGF-1 effects (after removal of IGF-1 resistance). We have not tested muscle strength during the therapy but it is thought that muscle function is improved during anti-TNF therapy as indicated by improved HAQ scores. Positive effects of TNF-neutralising strategies on growth rates have been described in children with juvenile idiopathic arthritis. In addition, positive effects of improved IGF-1 signaling on TNF-induced cartilage degradation are also expected.

Our two patients groups with and without glucocorticoid therapy were different in age, which may have influenced our results. Patients receiving prednisolone were on average 10 years older than patients without exogenous glucocorticoids. Since IGF-1 levels decrease during aging, one would have expected lower IGF-1 serum levels in aged patients (those with prednisolone). However, we observed exactly the opposite phenomenon of increased IGF-1 levels in these patients, which additionally supports the idea of IGF-1 resistance.

Furthermore, the observed effects of anti-TNF are most probably independent on binding proteins of IGF-1 because serum levels of IGFBP-1 and IGFBP-2 did not change during therapy. In this study, we observed a positive interrelation between serum IL-6 and serum IGFBP-1, which confirms a positive influence of IL-6 on synthesis of these binding proteins.

In conclusion, this study seems to support an attenuation of IGF-1 resistance in RA patients with prednisolone therapy treated with anti-TNF-antibodies. These findings likely demonstrate important new favourable effects of anti-TNF therapy in patients with RA treated with corticosteroids. Future studies in larger samples over a longer period of time may address whether, or not, anti-TNF therapy increases muscle mass as possibly measured by magnetic resonance imaging and muscle strength by clinical tests.

Acknowledgements
The respective institutions and Abbott S.p.A., Campoverde di Aprilia, Italy supported this study. We thank Angelika Gräber for excellent technical assistance.

Conflict of interest
There is no conflict of interest.
References


Table 1  Characteristics of patients under investigation. Data are given as means ± SEM and percentages in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>all patients</th>
<th>patients without glucocorticoids</th>
<th>patients with glucocorticoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>32</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>59.6 ± 2.1</td>
<td>55.2 ± 2.8*</td>
<td>64.0 ± 2.8</td>
</tr>
<tr>
<td>Gender (f / m)</td>
<td>30 / 2 (93.8 / 6.25)</td>
<td>15 / 1 (93.8 / 6.25)</td>
<td>15 / 1 (93.8 / 6.25)</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>6.9 ± 1.1</td>
<td>5.9 ± 1.6</td>
<td>7.9 ± 1.5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.5 ± 0.6</td>
<td>22.8 ± 0.8</td>
<td>22.3 ± 0.8</td>
</tr>
<tr>
<td>Baseline erythrocyte sedimentation rate (mm 1st hour)</td>
<td>27.7 ± 3.0</td>
<td>30.6 ± 4.8</td>
<td>24.8 ± 3.7</td>
</tr>
<tr>
<td>Baseline C-reactive protein (mg / l)</td>
<td>14.5 ± 28.9</td>
<td>13.1 ± 4.3</td>
<td>15.9 ± 4.0</td>
</tr>
<tr>
<td>Baseline serum IL-6 (pg/ml)</td>
<td>27.7 ± 7.1</td>
<td>21.6 ± 7.4</td>
<td>33.9 ± 12.2</td>
</tr>
<tr>
<td>Positive for rheumatoid factor</td>
<td>30 (93.8)</td>
<td>15 (93.8)</td>
<td>15 (93.8)</td>
</tr>
<tr>
<td>Positive for antinuclear antibodies</td>
<td>2 (6.25)</td>
<td>1 (6.25)</td>
<td>1 (6.25)</td>
</tr>
<tr>
<td>Baseline swollen joint score (points)</td>
<td>8.9 ± 0.4</td>
<td>8.8 ± 0.7</td>
<td>9.1 ± 0.6</td>
</tr>
<tr>
<td>Baseline tender joint score (points)</td>
<td>10.0 ± 0.5</td>
<td>10.5 ± 0.8</td>
<td>9.5 ± 0.5</td>
</tr>
<tr>
<td>Baseline DAS28 (points)</td>
<td>5.5 ± 0.1</td>
<td>5.6 ± 0.2</td>
<td>5.3 ± 0.2</td>
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</tbody>
</table>

Additional therapy

<p>| | | | |</p>
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<tr>
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<tr>
<td>Prednisolone</td>
<td>16 (50.0)</td>
<td>0 (0.0)</td>
<td>16 (100.0)</td>
</tr>
<tr>
<td>Mean daily prednisolone (mg)</td>
<td>2.3 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>30 (93.8)</td>
<td>15 (93.8)</td>
<td>15 (93.8)</td>
</tr>
<tr>
<td>Mean weekly methotrexate (mg)</td>
<td>8.0 ± 0.7</td>
<td>8.3 ± 0.9</td>
<td>7.7 ± 1.0</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>27 (84.4)</td>
<td>15 (93.8)</td>
<td>12 (75.0)</td>
</tr>
<tr>
<td>Chloroquine / hydroxychloroquine</td>
<td>3 (9.4)</td>
<td>1 (6.25)</td>
<td>2 (12.5)</td>
</tr>
</tbody>
</table>

*p < 0.01 vs patients with glucocorticoids. All other parameters were not different between patients with versus without glucocorticoid therapy. No patient received azathioprine, leflunomide, cyclosporin A, or sulfasalazine.
Table 2  Course of response parameters during 12 weeks of adalimumab therapy. Data of patients with glucocorticoid therapy are given in parentheses. Data are given as means ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>number of swollen joints*</th>
<th>number of tender joints*</th>
<th>pain patient’s global assessment*</th>
<th>IL-6 serum levels (pg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline</td>
<td>8.8 ± 0.7 (9.1 ± 0.6)</td>
<td>10.5 ± 0.8 (9.5 ± 0.5)</td>
<td>54.8 ± 4.5 (54.8 ± 3.7)</td>
<td>21.6 ± 7.4 (33.9 ± 12.2)</td>
</tr>
<tr>
<td>week 2</td>
<td>7.3 ± 0.9 (7.0 ± 0.7)</td>
<td>9.6 ± 0.7 (8.1 ± 0.5)</td>
<td>40.8 ± 3.5 (40.7 ± 5.3)</td>
<td>4.1 ± 1.8 (7.0 ± 1.8)</td>
</tr>
<tr>
<td>week 6</td>
<td>4.3 ± 0.9 (4.3 ± 0.6)</td>
<td>7.5 ± 0.80 (6.5 ± 0.7)</td>
<td>32.3 ± 3.8 (38.5 ± 3.8)</td>
<td>8.7 ± 5.3 (5.5 ± 2.1)</td>
</tr>
<tr>
<td>week 12</td>
<td>2.8 ± 0.5 (3.4 ± 0.6)</td>
<td>6.1 ± 0.5 (5.4 ± 0.7)</td>
<td>23.3 ± 3.7 (25.2 ± 4.7)</td>
<td>2.9 ± 0.9 (13.8 ± 8.5)</td>
</tr>
</tbody>
</table>

*p < 0.003 indicating a decrease as assessed by the Friedman test.
Figure legends

Figure 1 Course of myoglobin serum levels in patients with rheumatoid arthritis. Black symbols demonstrate data of patients with prednisolone therapy, whereas white symbols reflect data of patients without prednisolone. Data are given as means ± SEM. *p<0.05, **p<0.005, and ***p<0.001 for the difference of medians versus patients without prednisolone. The Friedman p-values demonstrate whether values changed during the therapy.

Figure 2 Serum levels of insulin-like growth factor – 1 (IGF-1) at baseline (panel A) and during the course of anti-TNF therapy with adalimumab (panel B). A) IGF-1 serum levels at baseline as given by box plots in patients with and without glucocorticoid treatment. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. B) IGF-1 serum levels during the course of anti-TNF therapy in patients without (white symbols) and with (black symbols) prednisolone. Data are given as means ± SEM. **p<0.01 and ***p<0.001 for the comparison of medians versus patients without prednisolone. The Friedman p-values demonstrate whether values changed during the therapy (i.e., decrease in glucocorticoid – treated patients).

Figure 3 The hypothalamus – pituitary – liver – muscle (HPLM) axis. Growth hormone – releasing hormone (GHRH) from the hypothalamus stimulates pituitary growth hormone (GH) release. GH stimulates IGF-1 production from the liver. Liver IGF stimulates muscle growth directly and by inducing muscular IGF-1 (1). TNF and exogenous glucocorticoids inhibit effects of liver IGF-1 and local IGF-1 on muscle growth (2). TNF and glucocorticoids increase protein degradation in the muscle. Consumption of liver IGF-1 and down-regulation of local IGF-1 decrease the negative feedback signal to the liver (3). In patients with glucocorticoids, this leads to a loss of the negative feedback signal to the liver and, thus, to an increase of liver IGF-1 measurable in the serum. The question mark stands for other unknown muscular factors additionally involved in the negative feedback to the liver. IGF-1 itself inhibits GHRH release (4), which is also inhibited by endogenous and exogenous glucocorticoids.
Sarzi-Puttini et al., Fig. 1

![Graph showing serum myoglobin levels over time with and without glucocorticoids.](http://ard.bmj.com/)

- Without glucocorticoids: $p_{\text{Friedman}} = 0.65$
- With glucocorticoids: $p_{\text{Friedman}} = 0.39$
Sarzi-Puttini et al., Fig. 2

A

Serum IGF-1 (µg/l) vs. with or without glucocorticoids

B

Serum IGF-1 (µg/l) over time (weeks)

- pFriedman = 0.76
- pFriedman = 0.006

** p < 0.001

### n.s.
TNF inhibits IGF-1 signalling and increases protein degradation
Exogenous glucocorticoids stimulate myostatin and increase protein degradation

Sarzi-Puttini et al., Fig. 3
Anti-TNF antibody therapy improves glucocorticoid-induced insulin-like growth factor-1 (IGF-1) resistance without influencing myoglobin and IGF-1 binding proteins 1 and 3

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