Serum cortisol reduction and abnormal prolactin and CD4<sup>+</sup>/CD8<sup>+</sup> T-cell response as a result of controlled exercise in patients with rheumatoid arthritis and systemic lupus erythematosus despite unaltered muscle energetics


Objective. To investigate muscle energetics in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) and measure serum cortisol, prolactin and CD4<sup>+</sup>/CD8<sup>+</sup> T-cell levels during and after controlled exhaustive exercise.

Methods. Patients with RA (n = 7), patients with SLE (n = 6) and healthy individuals (HI) (n = 10) performed incremental cycle ergometry to the limit of tolerance. Ventilation, oxygen uptake (VO<sub>2</sub>) and carbon dioxide output were measured and the lactate threshold (LT) was estimated. Serum cortisol, prolactin, CD4<sup>+</sup> and CD8<sup>+</sup> lymphocyte subset levels were determined at baseline, peak exercise and 1 h after exercise.

Results. Exercise tolerance was reduced in patients with RA and patients with SLE, as reflected by peak VO<sub>2</sub> and LT, but muscle energetics were not altered. In RA and SLE, there was significant reduction in cortisol levels at peak (–10%; P = 0.03) and post-exercise times (–36%; P = 0.05). Prolactin varied significantly at peak exercise in HI only (+60%; P = 0.05). There was a significant reduction in CD4<sup>+</sup> T cells at peak exercise in RA (–15%; P = 0.02) and SLE patients (–8%; P = 0.04) and an increase after exercise in SLE patients (+11%; P = 0.03). In HI, CD8<sup>+</sup> T cells increased significantly (+47%; P = 0.01) at peak exercise, but this was not found in RA and SLE patients. A significant reduction in CD8<sup>+</sup> T cells was noted after exercise in SLE patients (–6%; P = 0.05).

Conclusion. RA and lupus patients do not have significantly altered muscle energetics, but have abnormal cortisol, prolactin and CD4<sup>+</sup>/CD8<sup>+</sup> T-cell responses to exercise. Further studies need to be carried out to evaluate whether short bouts of strenuous exercise have detrimental clinical effects.

Key words: Exercise, Rheumatoid arthritis, Systemic lupus erythematosus, Prolactin, Cortisol, CD4<sup>+</sup>/CD8<sup>+</sup> T cells.

For many years, the general opinion was that physical exercise is detrimental to patients with rheumatoid arthritis (RA) and may cause increased disease activity and further joint destruction. The effects of exercise on the hormonal and immune systems in RA have recently been reviewed [1] and it is apparent that exercise probably has no significant effect on disease activity [2]. One study even reported that regular training might actually slow the rate of radiological joint deterioration in RA [3].

Regular exercise plays an important part in the rehabilitation of patients with autoimmune rheumatic disease, significantly improving aerobic capacity, the time taken to walk 50 feet, depression, anxiety and the ability to perform normal activities of daily living [4, 5]. The mechanisms of these beneficial effects are poorly understood. However, it is recognized that the immunological and hormonal systems have close inter-regulatory links [6] and marked changes are seen in indexes of both immune and endocrine function in response to physical exercise. For example, in healthy individuals (HI), exercise leads to a rise in interleukin (IL) 1β, IL-6 and tumour necrosis factor [7]. IL-1β interacts with the endocrine system at the hypothalamic level, causing the secretion of corticotrophin releasing hormone (CRH) [8], and IL-6 has been shown to stimulate prolactin (PRL) release in animals [9].

In HI, cortisol levels rise during exercise of both moderate and severe intensity, and fall during recovery. The magnitude of the increase is similar in fit and untrained subjects at maximum exercise. Consequently, at a given work rate there is a greater increase in cortisol in the untrained. Exercise also induces secretion of other anterior pituitary hormones. For example, PRL and growth hormone levels have been reported to increase by as much as 230 and 2000% respectively in response to exhaustive physical exercise [10].

Glucocorticoids are anti-inflammatory hormones and play an important role in the treatment of autoimmune rheumatic disease. Endogenous glucocorticoids also appear to have a clinically therapeutic effect, disease activity correlating with the levels of serum cortisol throughout a 24-h period [11, 12].

PRL is a powerful proinflammatory peptide and its presence is crucial for the development of a number of experimental autoimmune diseases [13]. High levels of PRL are associated with clinical activity and the timing of disease onset in RA [14–16].
Significantly higher basal levels of PRL have also been described in certain patients with systemic lupus erythematosus (SLE) [17], and there appears to be a correlation with clinical and serological disease activity. Inadequately low serum levels of adrenocorticotropic hormone and cortisol have been described in patients with early RA and reactive arthritis [18]. However, no alterations of serum levels of adrenal and gonadal hormones in patients with ankylosing spondylitis have been observed [19]. Patients with RA have also been reported to have an abnormally low cortisol response to major surgery when compared with patients with osteoarthritis (OA) or osteomyelitis [20]. The RA patients showed a significantly greater increase in PRL when compared with the other two patient groups [21]. However, a more recent study reports insignificant increases in cortisol and PRL levels in both OA and RA patients following surgery [22].

We hypothesized that, in response to physical exercise performed to the limit of tolerance, patients with RA may show a subnormal response in cortisol secretion and an exaggerated rise in PRL. In addition, given the increased metabolic (and hence catabolic) rate associated with exercise, cortisol breakdown may outstrip secretion, leading to a fall in the level of the hormone. SLE patients were included in the study as relevant disease controls. Measuring oxygen uptake (VO2) and carbon dioxide output (VCO2) and estimating the subjects’ lactate threshold (LT) enabled us to uniquely control the experiment and make valid comparisons between these two diseases and HI with regard to exercise tolerance.

Methods

Patients and controls

All individuals taking part in the study were female Caucasians and did not undertake regular strenuous exercise. Patients with RA (average age 35 yr, range 20–44 yr, n = 7) and patients with SLE (average age 33 yr, range 22–37 yr, n = 6) were selected for the study from the rheumatology clinic at St George’s Hospital, London, on the basis of their ability to carry out exercise. Both groups of patients had active disease, as defined by serology, and satisfied American College of Rheumatology classification criteria [23, 24]. The patients had either never taken or had been off corticosteroids for at least 2 yr. All RA patients were taking methotrexate and a non-steroidal anti-inflammatory drug (NSAID) and three of the lupus patients were taking hydroxychloroquine. Patients who had received drugs that were likely to affect the PRL level (e.g. phenothiazines) were excluded. HI (n = 10) were recruited from hospital staff. Exercise testing was carried out between 9.00 and 12.00 h, and blood was taken from each subject prior to exercise, at peak exercise and 1 h after exercise. Local Ethics Committee approval was obtained.

Exercise test

All subjects were asked to refrain from alcohol and caffeine intake for the 24 h prior to the test and all tests were performed mid-morning in order to reduce the effects of circadian variability. The exercise test comprised a 3-min control period of unloaded pedalling, followed by an incremental ramp on a cycle ergometer at a rate of 10 W per min to the limit of subject tolerance [25].

Physiological analysis during exercise

During the test, expiratory airflow was determined from a pneumotachograph and strain-gauge which allowed peak VO2 and peak VCO2 to be measured using rapidly responding gas analysers for partial pressures of O2 (Zirconia fuel cell) and CO2 (infrared analyser), which were calculated using standard algorithms [26]. The highest level of VO2 achieved during the test (typically the final 15 s of the bout) was taken as the peak VO2 and the LT was estimated using the V-slope technique [27] and the ventilatory equivalents for O2 and CO2 [28]. The appropriateness of the ventilatory response to exercise was determined by the ventilatory equivalents for O2 and CO2 at the LT and at peak VO2, and also from the subthreshold slope of ventilation as a function of CO2 output.

Cortisol and PRL analysis

Cortisol was analysed using the Autodelfia kit (Wallac, Turku, Finland). This is an automated, solid-phase, time-resolved fluoro-immunoassay based on the competitive binding of europium-labelled cortisol. The level of cortisol is inversely proportional to the degree of immunofluorescence. The intra-assay and total precision were as shown in Table 1.

PRL was measured by an automated assay (Abbott UK) based on a microparticle enzyme immunoassay. Submicron-sized latex particles were coated with an antibody specific for PRL. The intra-assay and total precision were as shown in Table 2.

CD4 and CD8 lymphocyte subset analysis

The numbers of CD4+ and CD8+ T lymphocytes were measured by flow cytometry using a FACscan (Becton Dickinson, Oxford UK). The anti-CD4 and anti-CD8 antisera were obtained from Becton Dickinson, and the results are expressed as a percentage of the total lymphocyte gate.

Statistics

Differences in levels of cortisol, PRL, CD4 and CD8 T cells were calculated by measuring the percentage increase from base to peak and from base to 1 h after exercise. The differences were tested with a paired Wilcoxon test. The differences between groups were then tested using Kruskal–Wallis one way analysis of variance by ranks.

### Table 1. Intra-assay and total precision of measured cortisol levels

<table>
<thead>
<tr>
<th>nmol/l</th>
<th>Intra-assay</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>208</td>
<td>1.3</td>
<td>1.6</td>
</tr>
<tr>
<td>562</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>1268</td>
<td>1.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Analytical sensitivity <1 nmol/l. Functional sensitivity <8 nmol/l (cortisol level at which the inter-assay coefficient of variation is 20%).

### Table 2. Intra-assay and total precision of measured PRL levels

<table>
<thead>
<tr>
<th>mU/l</th>
<th>Intra-assay</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>2.8</td>
<td>3.6</td>
</tr>
<tr>
<td>657</td>
<td>2.5</td>
<td>4.1</td>
</tr>
<tr>
<td>2332</td>
<td>3.4</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Analytical sensitivity (zero calibrator ± 2 s.d.), 10 IU/l. The imprecision is very low and hence the functional sensitivity will equal the analytical sensitivity.
This was done because the variability was so different in the different groups. \( P < 0.05 \) was considered statistically significant.

**Results**

*Exercise analysis (Fig. 1)*

While exercise tolerance, as reflected by peak VO\(_2\) and the LT, was reduced in patients with RA and SLE compared with both controls and the predicted values for normal sedentary subjects using the prediction equations of Wasserman *et al.* [26], LT as a fraction of the peak VO\(_2\) was no higher in the patient groups than in the control group and was not significantly different between the patient groups.

None of the ventilatory response indices were significantly different between the patient groups and the control subjects. That is, the subthreshold slope of ventilation as a function of CO\(_2\) output was not significantly different, and neither was the proportional increase in ventilation above the LT. Naturally, as the control subjects exercised to higher work rates, both the maximum ventilation and VO\(_2\) were greater than the values in the patients in absolute terms, although the slope of the VO\(_2\) response with respect to the work (i.e. VO\(_2\)/watt) rate was not significantly lower in the patient groups (RA, 13.1 ± 1.9; SLE, 10.9 ± 1.2) compared with that of the controls (12.8 ± 1.7).

**Cortisol analysis (Table 3)**

The mean baseline values of cortisol were 310, 316 and 352 nmol/l for the HI, RA and SLE groups respectively. The resting levels of cortisol were not significantly different among the three groups. Six out of 10 HI, 1/7 RA patients and no SLE patients showed a rise in cortisol above the baseline value.

The HI showed a mean increase of 26% at peak exercise, and the increase was sustained at 22% above baseline 1 h after exercise. These increases did not reach statistical significance. In the RA patients at peak exercise there was a 10% reduction below the baseline value, and this was significantly reduced to 21% \((P = 0.04)\) 1 h after exercise. In SLE patients there was a significant reduction (by 15% \(P = 0.03\)) at peak exercise, and a significant further reduction below the baseline (36% \(P = 0.05\)) 1 h after exercise. At peak exercise, the reduction in cortisol levels was significantly greater in the SLE patients \((P = 0.05)\) than in the RA patients.

**Prolactin analysis (Table 3)**

The baseline mean PRL levels were 220, 226 and 310 mU/l for the HI, RA and SLE groups respectively. The resting levels of PRL in all three groups were not significantly different from each other.

The exercise resulted in an increase in PRL level above the baseline in 7/10 HI, 3/7 RA patients and 3/6 SLE patients.
The HI showed a significant increase of 66% ($P = 0.05$) at peak exercise, and this was reduced to around baseline levels 1 h after exercise. Although there were increases above baseline levels at peak exercise in the both the RA and the SLE group, with similar reductions (by 11 and 14% respectively) 1 h after exercise, these changes did not reach statistical significance. In addition, there was no difference in PRL fluctuations between the RA and lupus groups at any other time points.

**CD4$^+$ lymphocytes (Table 3)**

Baseline mean CD4 levels (percentage of total lymphocytes) were 38.9, 48.1 and 42.0 for the HI, RA and SLE groups respectively. The resting levels of CD4$^+$ lymphocytes in the three groups were not significantly different from each other.

The HI showed a reduction of 14% at peak exercise, reversing to 30% above baseline 1 h after exercise. These changes, however, were not statistically significant. RA patients, however, showed a significant reduction at peak exercise (by 15%; $P = 0.02$), reverting to approximately baseline values 1 h after exercise. In a similar fashion, lupus patients showed a significant reduction in values at peak exercise (by 8%; $P = 0.04$), but, in a similar fashion to HI, a significant increase above baseline (by 11%; $P = 0.03$) 1 h after exercise. There were no differences between the RA and SLE groups at any of these time points.

**CD8$^+$ lymphocytes (Table 3)**

The baseline mean CD8 levels (percentage of total lymphocytes) were 27.8, 27.4 and 27.9 for the HI, RA and SLE groups respectively. Resting levels of CD8 in all three groups were not significantly different from each other.

In HI, in contrast to CD4$^+$ cells, there was a significant increase (by 47%) at peak exercise ($P = 0.01$). This reduced at 1 h after exercise. In RA patients, there were slight fluctuations above baseline at peak exercise and below baseline 1 h after exercise; these changes did not reach statistical significance. Similarly, in lupus patients there was a slight increase above baseline at peak exercise, but a significant reduction (by 6%; $P = 0.05$) 1 h after exercise. There was no difference between RA and lupus patients at any of these time points.

**CD4$^+$/CD8$^+$ lymphocyte ratios (Table 3)**

The baseline mean CD4$^+$/CD8$^+$ ratios were 1.5, 1.9 and 1.5 for the HI, RA and SLE groups respectively. Resting levels of CD4/CD8 ratios in all three groups were not significantly different from each other.

In HI there was a reduction of 37% in the ratio ($P = 0.009$) at peak exercise, and although there was an increase above baseline 1 h after exercise, this did not reach statistical significance. In RA there was a significant reduction at peak exercise (by 18%; $P = 0.02$), with a return to approximate baseline values 1 h after exercise. In SLE patients, at peak exercise there was a 9% reduction, which was not significant, whereas 1 h after exercise there was a significant increase (by 18%; $P = 0.03$) 1 h after exercise. At peak exercise the reduction in RA patients was significantly greater than that in lupus patients ($P = 0.03$).

**Discussion**

The aims of this study were (i) to compare exercise tolerance and the physiological mechanisms that couple O$_2$ utilization to work rate or ventilation to metabolic rate in SLE and RA patients with corresponding values in HI, and (ii) to investigate longitudinal cortisol, PRL and CD4$^+$/CD8$^+$ T-cell responses to controlled and predefined exhaustive exercise in these groups.

Although exercise tolerance was reduced both in patients with RA and in patients with SLE, there was no evidence that lack of effort was the cause, as the estimated lactate threshold (as a fraction of the VO$_2$ peak) was not different among groups. In addition, there was no evidence that disease processes affected the fundamental physiological mechanisms that couple O$_2$ utilization to work rate and/or couple ventilation to metabolic rate. The slope of the VO$_2$/work rate relationship, as an index of work efficiency [25], was not less than that in our control group or values reported previously, which cover a wide age range [24, 27, 29]. This relationship remained linear following the early lag associated with the response time constant, up to the maximum tolerable work rate. It did not become shallower at high work rates, as seen in patients with heart disease [26], and was not shallower than normal throughout, as seen in patients with peripheral vascular disease [26]. This suggests that the diseases did not impair muscle energetics *per se* and that the low peak VO$_2$ and LT (Fig. 1 and Table 3) were probably consequences of the restricted activity patterns of the patients. However, we were not able to consider other details of muscular function, such as the kinetics of O$_2$ uptake [30, 31].

There were both endocrine and immunological fluctuations associated with controlled exhaustive exercise that were found only in the autoimmune rheumatic disease groups. In contrast to

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**Table 3. Mean percentage (s.d.) changes in cortisol, PRL, CD4$^+$ and CD8$^+$ T cells and CD4$^+$/CD8$^+$ T-cell ratio in control (HI), RA and SLE groups**

<table>
<thead>
<tr>
<th></th>
<th>Control ($n=10$)</th>
<th>RA ($n=7$)</th>
<th>SLE ($n=6$)</th>
<th>Disease groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortisol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to peak</td>
<td>Mean (s.d.)</td>
<td>P</td>
<td>Mean (s.d.)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>26 (70)</td>
<td>0.9</td>
<td>-10 (23)</td>
<td>0.2</td>
</tr>
<tr>
<td>Base to 1 h after exercise</td>
<td>22 (78)</td>
<td>0.9</td>
<td>-21 (18)</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td><strong>Prolactin</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Base to peak</td>
<td>Mean (s.d.)</td>
<td>P</td>
<td>Mean (s.d.)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>66 (147)</td>
<td><strong>0.05</strong></td>
<td>3 (23)</td>
<td>0.9</td>
</tr>
<tr>
<td>Base to 1 h after exercise</td>
<td>2 (44)</td>
<td>0.3</td>
<td>-11 (25)</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>CD4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to peak</td>
<td>Mean (s.d.)</td>
<td>P</td>
<td>Mean (s.d.)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>-14 (39)</td>
<td>0.09</td>
<td>-15 (7)</td>
<td><strong>0.02</strong></td>
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<tr>
<td>Base to 1 h after exercise</td>
<td>30 (88)</td>
<td>0.2</td>
<td>-2 (12)</td>
<td>0.7</td>
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<tr>
<td><strong>CD8</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to peak</td>
<td>Mean (s.d.)</td>
<td>P</td>
<td>Mean (s.d.)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>47 (81)</td>
<td><strong>0.01</strong></td>
<td>6 (20)</td>
<td>0.4</td>
</tr>
<tr>
<td>Base to 1 h after exercise</td>
<td>16 (72)</td>
<td>0.5</td>
<td>-8 (12)</td>
<td>0.1</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to peak</td>
<td>Mean (s.d.)</td>
<td>P</td>
<td>Mean (s.d.)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>-37 (24)</td>
<td><strong>0.009</strong></td>
<td>-18 (15)</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>Base to 1 h after exercise</td>
<td>13 (21)</td>
<td>0.1</td>
<td>9 (23)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Differences between the groups were analysed with the paired Wilcoxon test and the Kruskal–Wallis test. Significant changes are indicated in bold.
the cortisol increase in the healthy population, significant reductions in cortisol were noted at both peak and after exercise in both the RA and the lupus group. Indeed, in the lupus group at peak exercise this reduction was significantly greater than that seen in the RA population. In the healthy population, a significant increase in PRL was noted at peak exercise, whereas no significant fluctuations were noted in either the RA or the lupus disease group.

A reduction in CD4⁺ T cells was noted at peak exercise in the healthy group and the RA and lupus disease groups, but this reduction was only of significance in the disease groups. An increase in CD4⁺ T cells was noted in all groups 1 h after exercise, but this only reached significance in the lupus group. A significant increase in CD8⁺ T cells was noted in the healthy group at peak exercise and a trend in that direction was noted in the lupus and RA groups. A significant rebound reduction was noted after exercise in the lupus group. These changes were reflected in CD4/CD8 ratio variations, with negative changes at peak exercise in healthy and RA groups and a rebound positive change after exercise in the lupus group.

CD4⁺ T cells secrete cytokines that help activate other types of immune system cells and respond to peptide antigens presented by MHC class II antigens. CD8⁺ T cells have a cytotoxic function and are also known as cytotoxic T cells. Effector CD8⁺ T cells kill cells infected with viruses and other intracellular pathogens. This prevents the spread of infection. The sequela to exercise-induced CD4⁺/CD8⁺ T-cell fluctuations would therefore depend upon whether the cells affected were autoreactive and for how long the fluctuations were to last. Longer-term and functional studies are therefore required to address this [32–34]. However, such studies may be impossibly complex as not only will there be patient-specific variability, involving such things as age, sex, ethnicity and medication, but the complexities of lymphocyte trafficking and the associated influences of heat stress, surface glycoprotein expression, together with the cytokine and hormonal environment, will also need to be considered [32–34].

This study shows that there are similarities in the manner in which cortisol levels change in RA and SLE patients when undertaking exercise of this nature. Although there are differences between these results and those from post-surgery studies [20], in which only a small increase was observed in RA patients, both studies show that there is an inadequate cortisol response to a stressful event.

The cause of bluntness of the cortisol response to surgery in RA is thought to lie at the hypothalamic level, with failure to secrete adequate CRH [20]. Cytokines are thought to activate the hypothalamus–pituitary–adrenal (HPA) axis at the hypothalamus, via the prostaglandin pathway [35–37]. Drugs such as NSAIDs, which interfere with prostaglandin synthesis, are likely to have an inhibitory effect on CRH release, and hence cortisol production. In both studies [20, 22], NSAID use was widespread, which may well explain why there was no significant increase in the OA patients in the latter study, but it would not explain the differences between the two studies. To clarify whether RA affects the HPA axis directly, the studies would have to be repeated in patients not taking corticosteroids, disease modifying anti-rheumatic drugs or NSAIDs.

Given the relationship between endogenous cortisol secretion and disease activity, an exercise of this nature has the potential to cause a transient increase in disease activity, particularly if steroid levels remain suppressed for many hours. Patients who have been on corticosteroids or NSAIDs for long periods have further factors causing HPA axis suppression and are therefore likely to be more severely affected. The higher metabolic rate of exercise is also likely to lead to an increase in the catabolism of ingested steroids. Whether or not medication change is necessary prior to strenuous exercise has yet to be determined.

We also demonstrated that baseline PRL levels of RA and SLE patients and HI were not significantly different. At peak exercise, small and insignificant increases in PRL were seen in both SLE and RA patients. The HI, in keeping with previous studies, showed a large PRL increase at peak exercise. The data showed no evidence for an exaggerated PRL increase in RA, and suggest that the PRL response may even be subnormal in RA and SLE.

RA and lupus patients do not have significantly altered muscle energetics, but have abnormal cortisol, PRL and CD4⁺ and/or CD8⁺ T-cell responses to exercise. Further studies need to be carried out to evaluate whether short bouts of strenuous exercise have detrimental clinical effects.

References