

# Acute cold stress in rheumatoid arthritis inadequately activates stress responses and induces an increase of interleukin 6

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## ABSTRACT

**Objective:** Acute stress in patients with rheumatoid arthritis (RA) should stimulate a strong stress response. After cryotherapy, we expected to observe an increase of hormones of the adrenal gland and the sympathetic nervous system.

**Methods:** A total of 55 patients with RA were recruited for whole-body cryotherapy at  $-110^{\circ}\text{C}$  and  $-60^{\circ}\text{C}$ , and local cold therapy between  $-20^{\circ}\text{C}$  and  $-30^{\circ}\text{C}$  for 7 days. We measured plasma levels of steroid hormones, neuropeptide Y (sympathetic marker), and interleukin (IL)6 daily before and after cryotherapy.

**Results:** In both therapy groups with/without glucocorticoids (GC), hormone and IL6 levels at baseline and 5 h after cold stress did not change over 7 days of cryotherapy. In patients without GC, plasma levels of cortisol and androstenedione were highest after  $-110^{\circ}\text{C}$  cold stress followed by  $-60^{\circ}\text{C}$  or local cold stress. The opposite was found in patients under GC therapy, in whom, unexpectedly,  $-110^{\circ}\text{C}$  cold stress elicited the smallest responses. In patients without GC, adrenal cortisol production increased relative to other adrenal steroids, and again the opposite was seen under GC therapy with a loss of cortisol and an increase of dehydroepiandrosterone. Importantly, there was no sympathetic stress response in both groups. Patients without GC and  $-110^{\circ}\text{C}$  cold stress demonstrated higher plasma IL6 compared to the other treatment groups (not observed under GC), but they showed the best clinical response.

**Conclusions:** We detected an inadequate stress response in patients with GC. It is further shown that the sympathetic stress response was inadequate in patients with/without GC. Paradoxically, plasma levels of IL6 increased under strong cold stress in patients without GC. These findings confirm dysfunctional stress axes in RA.

Acute stress studies in patients with rheumatoid arthritis (RA) reported inadequate adrenocorticotropic hormone (ACTH)/cortisol release after insulin-induced hypoglycaemia and during a corticotropin releasing hormone test,<sup>1-3</sup> but these studies are inconclusive.<sup>4</sup> Unconvincing results may depend on the inflammatory status prior to or at the time point of the test, which has not been adequately controlled or reported. Furthermore, it may be that relatively strong stress stimuli such as insulin hypoglycaemia do not reveal subtle alterations of the hypothalamic-pituitary-adrenal (HPA) axis, and only acute minor stress may reveal dysfunctions of these hormonal pathways. Indeed, Dekkers *et al* have demonstrated that patients with

RA do not mount a significant ACTH response upon controlled psychological stress, which is also visible in form of inadequate cortisol secretion during the test phase.<sup>5</sup> In addition, it has been demonstrated that controlled exercise-induced release of cortisol decreased in patients with RA as compared to controls.<sup>6</sup> Controlled adrenaline infusion, simulating a stress response, leads to a fast decrease of cortisol serum levels in RA but not in controls.<sup>7</sup> Although the HPA axis is relatively robust, it seems that acute mild to moderate stress can lead to an unexpected decrease of HPA axis responses in patients with RA. This would yield an overall proinflammatory situation in the chronic symptomatic phase of the disease. A further question arises in patients with RA how the other stress system, the sympathetic nervous system (SNS), is activated under acute stress.

Several studies have demonstrated an increased baseline sympathetic tone in patients with RA and juvenile idiopathic arthritis (JIA).<sup>8-12</sup> On the basis of an increased sympathetic tone, the sympathetic response to acute stressful situations such as tilting<sup>10</sup> or psychological testing<sup>13</sup> generated reduced SNS responses or increased responses.<sup>8</sup> In RA and JIA, this may demonstrate the general inability of the SNS to adequately adapt to necessary needs under stressful conditions. Similarly, patients with systemic sclerosis did not mount an adequate stress response of the SNS because plasma norepinephrine did not rise after insulin hypoglycaemia.<sup>14</sup> Similarly to the HPA axis, one would expect that an increased basal sympathetic tone and a loss of sympathetic activation during stress would support proinflammatory responses (reviewed in Straub *et al*).<sup>15</sup> These studies prompted us to investigate the role of cold stress as applied during cryotherapy on hormones of the HPA axis and the SNS.

Cold stress such as whole-body immersion in cold water,<sup>16</sup> a stay in the Arctic or Antarctic,<sup>17 18</sup> or hand or foot immersion in cold water<sup>19 20</sup> stimulates the SNS and the HPA axis. Thus, cold stress during controlled whole-body cryotherapy might be a good stress paradigm to test the activity of the HPA axis and the SNS in patients with RA. Whole-body cryotherapy in patients with RA was introduced for therapeutic purposes in the 1970s,<sup>21</sup> and we recently started to apply this method to Finnish patients with RA.<sup>22</sup> After 7 days of treatment, effects on disease activity were modest but whole-body cryotherapy at  $-110^{\circ}\text{C}$  seems to relieve pain.<sup>22</sup> In this study, we included

three different groups with whole-body cryotherapy at  $-110^{\circ}\text{C}$  and  $-60^{\circ}\text{C}$ , and with local cryotherapy applied to five swollen joints with cold packs or cold air.<sup>22</sup> It was thought that the investigation of hormonal and neuronal pathways before and after cryotherapy might shed some more light on cold stress-induced alterations of stress axes in patients with RA. Since glucocorticoid (GC) treatment can change hormonal and neuronal readout parameters, the entire patient group was separated according to ongoing prednisolone treatment. ACTH and adrenal steroid hormones were used to test the HPA axis, and neuropeptide Y (NPY) was used to study the SNS.<sup>12</sup>

## PATIENTS AND METHODS

### Study population

The study was performed between September 2000 and May 2003. A total of 55 patients were included who had active seropositive RA fulfilling the American College of Rheumatology (ACR) criteria.<sup>23</sup> Clinical variables of disease activity included the number of swollen and tender joints, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) (the latter two were measured by standard techniques). The influence of cryotherapy on clinical markers of inflammation was reported previously.<sup>22</sup> Basic characteristics of the study group, including therapy, are demonstrated in table 1. All patients without prednisolone had not received GC for a period of at least 1 month before study entry, whereas patients with prednisolone had stable low-dose glucocorticoid therapy over 1 month before study entry. The patients had no intra-articular GC injections within 1 month prior to the study. We were not able to include a control group without RA because a respective age-matched group was not available. All patients gave their written informed consent. The study protocol was approved by the Ethical Committee of Päijät-Häme Hospital district, Finland.

### Study design

On arrival, the patients were randomised to use traditional local cryotherapy (cold packs or cold air at  $-20^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  applied to five swollen joints at a time for 10–30 min), whole-body cryotherapy at  $-60^{\circ}\text{C}$  (for 2 min), or whole-body cryotherapy at  $-110^{\circ}\text{C}$  (for 2 min) three times daily at 8:00 am, 12:00 am and 4:30 pm. The treatment, with a total of 20 courses (6×3 courses on 6 working days and 1×2 courses on Sunday), was applied over 7 consecutive days. On a treatment day, blood

samples were taken before the first cryotherapy (baseline, 08:00) and after the second cryotherapy (13:00). Blood samples were taken daily during the 7-day treatment period. On the first day, blood samples were also taken 1 h after the first session of cryotherapy at 09:00. The blood was taken into 10 ml EDTA tubes in ice water and immediately centrifuged, and plasma was stored on  $-80^{\circ}\text{C}$ . The measured parameters are stable for a long period of time when stored on  $-80^{\circ}\text{C}$ .

### Laboratory parameters

Several adrenal hormones were considered in order to detect major adrenal pathways of steroidogenesis. We used radio-immunometric assays for the quantitative determination of plasma levels of cortisol (Coulter Immunotech, Marseilles, France; detection limit: 10 nmol/litre; cross-reactivity vs prednisone or prednisolone <6%) and plasma levels of NPY (Euro-Diagnostica AB, Malmö, Sweden; detection limit: 6 pmol/litre). Plasma levels of ACTH (Sangui BioTech, Santa Ana, California, USA; detection limit: 0.1 pmol/litre), androstenedione (ASD; IBL, Hamburg, Germany; detection limit: 0.14 nmol/litre), 17-hydroxyprogesterone (17OHP; IBL; detection limit: 0.3 nmol/litre), dehydroepiandrosterone (DHEA; Diagnostic Systems Laboratory, Webster, Texas, USA; detection limit: 0.13 nmol/litre) and interleukin (IL)6 (high sensitivity Quantikine, R&D Systems, Minneapolis, Minnesota, USA; detection limit: 0.2 pg/ml) were measured by means of immunometric enzyme immunoassays.

Intra-assay and interassay coefficients of variation were below 10% in all tests.

### Calculation of stress-induced effects on readout parameters and hormone ratios

Since the stress was applied in the morning hours, the usual decline of steroid hormones had to be taken into account due to the circadian rhythm of hormones with a maximum at 08:00. We measured the baseline plasma level of a hormone at 08:00 (peak level) and determined the hormone level after two sessions of cryotherapy at 13:00. The value at 13:00 was expressed as percentage of the baseline value, as indicated in fig 1. Due to the variation of the response, the 13:00 value in percentage terms can be above 100% indicating an increase or below 100% representing a decrease in relation to the baseline level (fig 1). Due to the natural decline of hormones in the morning hours in a situation without cold stress, one would

**Table 1** Characteristics of patients in the study

|                             | Without glucocorticoids |                       |                        | With glucocorticoids |                       |                        |
|-----------------------------|-------------------------|-----------------------|------------------------|----------------------|-----------------------|------------------------|
|                             | Local cold              | $-60^{\circ}\text{C}$ | $-110^{\circ}\text{C}$ | Local cold           | $-60^{\circ}\text{C}$ | $-110^{\circ}\text{C}$ |
| Age, years                  | 59.3 (2.2)              | 45.4 (5.8)            | 50.6 (1.5)             | 57.5 (2.2)           | 55.7 (2.4)            | 50.6 (4.0)             |
| Sex, f/m                    | 7/3                     | 5/0                   | 7/2                    | 9/1                  | 10/2                  | 8/1                    |
| Disease duration, years     | 12.3 (3.0)              | 11.5 (4.8)            | 11.4 (3.7)             | 20.4 (3.0)           | 17.7 (3.6)            | 13.1 (3.4)             |
| Swollen joints, baseline, n | 19.7 (2.9)              | 19.2 (2.2)            | 16.4 (2.6)             | 17.6 (2.1)           | 13.2 (1.9)            | 14.9 (2.3)             |
| Tender joints, baseline, n  | 26.5 (3.5)              | 18.0 (3.9)            | 18.1 (2.8)             | 18.4 (3.9)           | 12.4 (2.3)            | 19.1 (3.5)             |
| ESR, baseline, mm first h   | 35.2 (4.2)              | 42.6 (11.5)           | 37.6 (4.8)             | 39.2 (5.2)           | 29.0 (4.2)            | 36.11 (7.5)            |
| CRP, baseline, mg/litre     | 21.8 (7.1)              | 37.4 (17.8)           | 19.9 (7.0)             | 28.6 (7.6)           | 28.7 (4.7)            | 32.2 (7.8)             |
| Medication:                 |                         |                       |                        |                      |                       |                        |
| Prednisolone, mg/day        | 0                       | 0                     | 0                      | 6.5 (0.7)            | 7.5 (1.1)             | 4.7 (0.5)              |
| DMARD (all types), n        | 7                       | 3                     | 6                      | 7                    | 11                    | 8                      |
| NSAID, n                    | 7                       | 5                     | 8                      | 8                    | 9                     | 8                      |

Data are given as mean (standard error of the mean (SEM)). In the group with glucocorticoids, daily prednisolone dose was not statistically different between groups.

CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; NSAID, non-steroidal anti-inflammatory drug.

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expect a 13:00 value clearly below 100% (fig 1). In a recent review, we determined the 13:00 cortisol value given in several studies with patients with RA and healthy controls to be between 60% to 70%.<sup>24</sup>

In order to demonstrate a shift from one plasma hormone to another plasma hormone, the molar ratio of these hormones was calculated (given without units, similar to Anderson and Yen, Barone *et al* and Nestler *et al*).<sup>25–27</sup> This procedure detects a hormonal shift through one or two adrenal enzyme steps, which can demonstrate a preponderance of an adrenal hormone pathway: cortisol/17OHP for the pathway through P450c21 and P450c11 into the direction of cortisol, ASD/17OHP for the 17,20 lyase (second reaction of the P450c17) into the direction of ASD, and cortisol/DHEA for the combined reaction of the 3 $\beta$ -hydroxysteroid dehydrogenase, and P450c17, P450c21 and P450c11 into the direction of cortisol. We used this method in earlier studies in order to demonstrate a hormonal pathway predominance (eg, Straub *et al*).<sup>28, 29</sup>

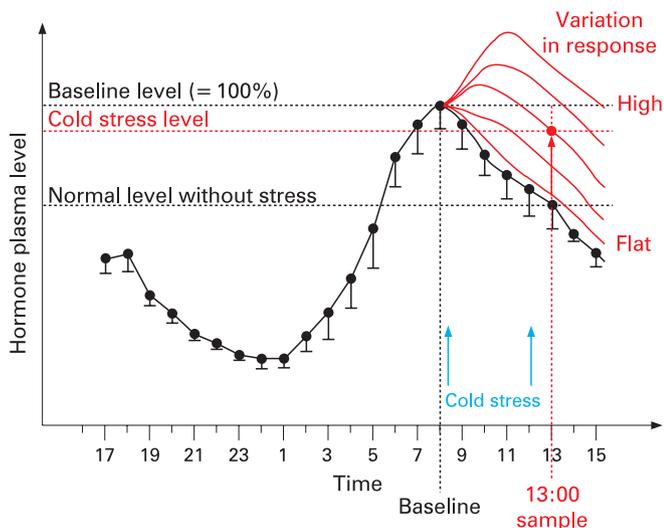
### Presentation of data and statistical analysis

The data are given as scatter plots (hormone level over time) or box plots with the 10th, 25th, 50th (median), 75th and 90th percentile. Group medians were compared by the non-parametric Mann–Whitney U test, and correlations were calculated by Spearman rank correlation analysis (SPSS V.15.0, SPSS, Chicago, Illinois, USA).  $p < 0.05$  was set as the level of significance.

## RESULTS

### The 08:00 and 13:00 hormone values over 7 days of cryotherapy

Initially, it was thought that 08:00 and 13:00 values might change during a 7-day course of repeated cryotherapy sessions, which might be a sign for the favourable adaptation of the HPA

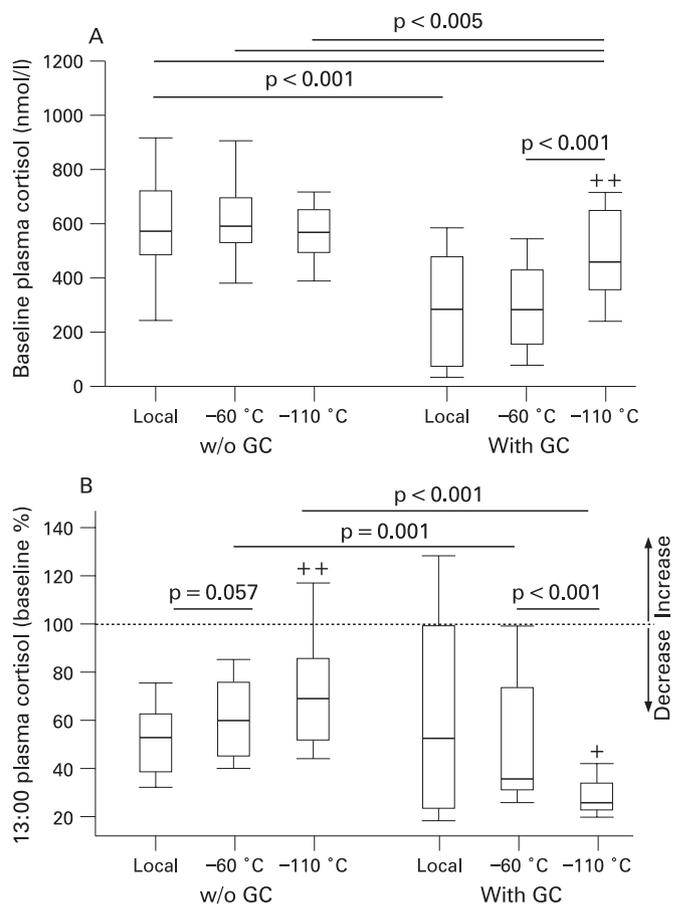


**Figure 1** The natural circadian rhythm of plasma cortisol. Change of the natural circadian rhythm of cortisol by cold stress in the morning. The baseline determination at 08:00 gives the peak value during the day. Cold stress was applied at 08:00 and 12:00 (blue arrows), which can lead to different response curves during the morning hours (given in red colours). In our calculations, the 13:00 value was expressed as percentage of baseline (at 08:00). This value in percentage terms can be above 100% (increase), exactly 100% (stable situation), or below 100% (decrease). The natural decline during the morning hours provides a 13:00 value of approximately 70% as substantiated in an earlier review on circadian rhythms in patients with rheumatoid arthritis and healthy controls.<sup>24</sup>

axis and the SNS. Such an adaptation might be represented by slowly increasing or decreasing baseline or 13:00 values. However, measured hormone levels at 08:00 (baseline) did not change during the course of the therapy (data not shown). Similarly, the plasma levels of hormones measured at 13:00 did not change during the course of cryotherapy (data not shown). As expected, plasma levels of cortisol were lower in patients receiving therapeutic GC (fig 2A). This was similar with respect to the other adrenal steroid hormones and ACTH (data not shown). This indicates the expected mild pituitary–adrenal inhibition in patients treated with GC, which justifies the demonstration of two different groups with respect to GC treatment.

### Effect of cryotherapy on 13:00 hormone levels relative to baseline hormone levels

As demonstrated in fig 1, the change between baseline and 13:00 may lead to an increase of hormones in the morning hours (the 13:00 value is above baseline, >100%), to a stable situation



**Figure 2** Baseline and 13:00 cortisol plasma levels of patients with rheumatoid arthritis (RA) with and without glucocorticoids (GC) under different degrees of cold stress. A. Baseline plasma cortisol in patients with and without GC under different degrees of cold stress. Since there were no differences between various days during cryotherapy (data not shown), the values of all patients and days were combined into one variable. B. 13:00 plasma levels (relative to baseline) in patients with and without GC under different degrees of cold stress (for the combined calculation see explanation in the legend to (A)). The dotted line in (B) indicates the 100% level. For both panels  $p < 0.01$ ,  $++p < 0.005$  vs local cold therapy in the respective treatment group. Box plots show the 10th, 25th, 50th (median), 75th and 90th percentile. w/o GC, without glucocorticoids; with GC, with glucocorticoids.

(= 100%), or to a decrease (<100%). With respect to cortisol, ACTH, 17OHP, ASD, DHEA and NPY, this cold stress-induced effect on plasma levels did not change over the 7 days of cryotherapy (data not shown). This indicates that the cold stress-induced effect on hormone secretion in the morning hours was not influenced by repeated cryotherapy.

Since this behaviour of the hormone level did not change over the 7 days of cryotherapy, the values of the 7 treatment days were combined into one variable for analysis. In patients without GC, the 13:00 levels of cortisol relative to baseline were highest under  $-110^{\circ}\text{C}$  cold stress followed by  $-60^{\circ}\text{C}$  or local cold stress (fig 2B). This indicates that patients undergoing  $-110^{\circ}\text{C}$  cold stress had the smallest decrease of cortisol levels during the morning hours (ie, they had the strongest stress response).

Importantly, the opposite of this cold stress-induced effect was observed in patients under GC therapy (fig 2B). In patients treated with GC, those patients with  $-110^{\circ}\text{C}$  cold stress in particular demonstrated a weak response of the adrenal glands, only reaching the level of approximately 25% of the baseline level (fig 2B). In the direct comparison of patients with vs without GC under  $-110^{\circ}\text{C}$  or  $-60^{\circ}\text{C}$  cold stress, a highly significantly different response was observed (fig 2B), which demonstrates cold stress-amplified adrenal insufficiency in patients treated with GC. Such a response was not observed for ACTH, where the different cold stress groups demonstrated very similar levels independent of GC therapy (data not shown).

#### Effect of cryotherapy on 13:00 hormone ratios relative to baseline hormone ratios

Since in patients without GC the  $-110^{\circ}$  cold stress leads to a less pronounced decrease of cortisol during the morning hours (fig 2B), it is expected that adrenal cortisol secretion is facilitated by an increase of adrenal cortisol relative to other adrenal hormones (the cortisol pathway becomes predominant relative to other adrenal steroid pathways).

While in patients without GC plasma levels of DHEA and 17OHP did not markedly change during the morning hours (data not shown), the ratio of plasma cortisol/plasma DHEA and plasma cortisol/plasma 17OHP showed a similar behaviour to cortisol alone (fig 3 vs fig 2B). In the  $-110^{\circ}\text{C}$  cold stress group without GC, the ratio of plasma cortisol/plasma DHEA exceeded the 100% line and the ratio of plasma cortisol/plasma 17OHP reached the 100% line (fig 3A,B). This was completely opposite to patients treated with GC (fig 3A,B). In patients with GC, the respective ratios of plasma cortisol/plasma DHEA and plasma cortisol/plasma 17OHP were smallest in the  $-110^{\circ}\text{C}$  group (fig 3A,B), which indicates that under GC therapy secretion of DHEA and 17OHP was increased at the expense of cortisol.

With respect to ASD and the ratio of plasma ASD/plasma 17OHP, no such changes were observed, which indicates that the second enzymatic step of the P450c17 was not involved in the above-mentioned effects (data not shown).

#### Effect of cryotherapy on plasma levels of NPY and IL6

Under cold stress, a typical increase of the activity of the SNS is expected.<sup>16 17 19</sup> Under consideration of a blood half-life for NPY of approximately 30 min<sup>30</sup> and plasma levels independent of the circadian rhythm,<sup>31</sup> one would expect an increase of plasma NPY shortly after a cold stress. However, we did not observe an increase or a fall of plasma NPY 1 h or 5 h after the first or second cryotherapy session, respectively (the level remained at

the 100% line; fig 4A,B). This was independent of GC administration (fig 4A,B).

In order to determine a cold stress-induced effect on an important parameter of immunological activation, we measured plasma levels of IL6. Importantly, in patients without GC, plasma IL6 remained high in the  $-110^{\circ}\text{C}$  cold stress group (near the 100% line), while it decreased under  $-60^{\circ}\text{C}$  or local cold stress (fig 4C). In the direct comparison of patients with and without GC under  $-110^{\circ}\text{C}$  cold stress, IL6 plasma levels decreased under GC therapy whereas it did not in patients without GC (fig 4C). Since IL6 undergoes a circadian rhythm similar to cortisol,<sup>24</sup> this indicates that IL6 remained elevated in the  $-110^{\circ}\text{C}$  group without GC (near the 100% line in fig 4C) compared to patients with GC (below the 100% line in fig 4C).

#### Effect of cryotherapy on clinical parameters

Since in patients without GC, 13:00 levels of cortisol relative to baseline were highest under  $-110^{\circ}\text{C}$  cold stress (fig 2B) but in the same group IL6 levels were also highest (fig 4C), the question arises whether or not these patients benefit from 7 days of cryotherapy. As demonstrated in fig 5, it is exactly this group with the best test results concerning swollen joint count (fig 5A) and pain score (fig 5B). This indicates that, despite elevated IL6 plasma levels, the patients in the  $-110^{\circ}\text{C}$  group without GC had a favourable outcome. In the other cold stress groups with and without GC, no significant improvement was observed. This indicates that  $-110^{\circ}\text{C}$  cold stress is different with respect to the beneficial effect compared to  $-60^{\circ}\text{C}$  or local cold stress.

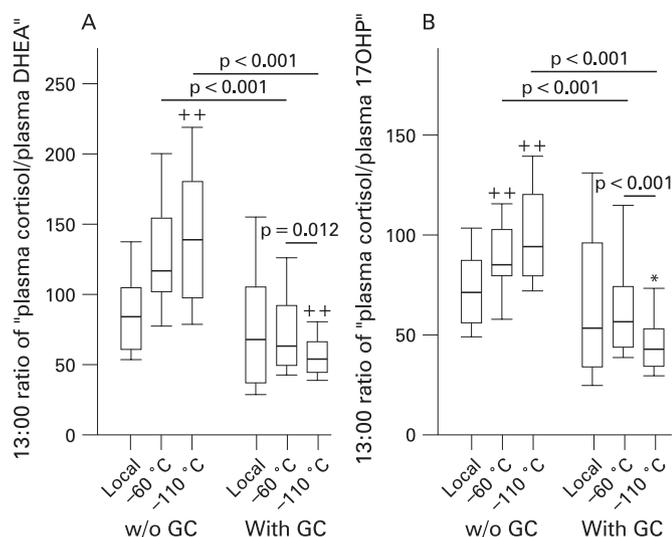
#### DISCUSSION

The HPA axis and the SNS have been shown to be vulnerable in patients with rheumatoid arthritis and other chronic inflammatory diseases.<sup>32 33</sup> A defect of stress axes may lead to unwanted proinflammatory side effects.<sup>15</sup> Cold stress as applied during cryotherapy might be a good stress paradigm to further study changes of the HPA axis and the SNS.

As expected,  $-110^{\circ}\text{C}$  cold stress led to the highest post stress cortisol levels compared to  $-60^{\circ}\text{C}$  and local cold stress. We were not able to compare this effect with an age-matched control group because no such group was available. However, several historical studies on the circadian rhythm of cortisol in patients with RA demonstrated that the 13:00 value is approximately 60% to 70% relative to the baseline level at 08:00 (peak level) (see fig 1; reviewed in Straub *et al.*).<sup>24</sup> Compared to this 70% level in non-stressed patients with RA and healthy subjects, the 13:00 levels in this study after cold stress are similar for the different cold stress groups ranging between 50% and 70%. From this point of view, cold stress in our study did not elicit a dramatic stress response, which should lead to 13:00 plasma concentrations above the 100% level (see fig 1). Importantly, patients under GC demonstrated a marked reduction of stress-induced secretion of cortisol, DHEA and 17OHP (particularly in the  $-110^{\circ}\text{C}$  group). This clearly indicates that the endogenous cortisol stress response is largely disturbed in patients under GC. One might use cold stress as a diagnostic tool to judge adrenal insufficiency, which needs to be investigated in prospective studies.

Since in patients without GC the  $-110^{\circ}\text{C}$  cold stress leads to the highest cortisol level during the morning hours as compared to the other cold stress groups, it is expected that cortisol secretion is facilitated by an increase of cortisol relative to other adrenal hormones such as DHEA or 17OHP. Such a pathway

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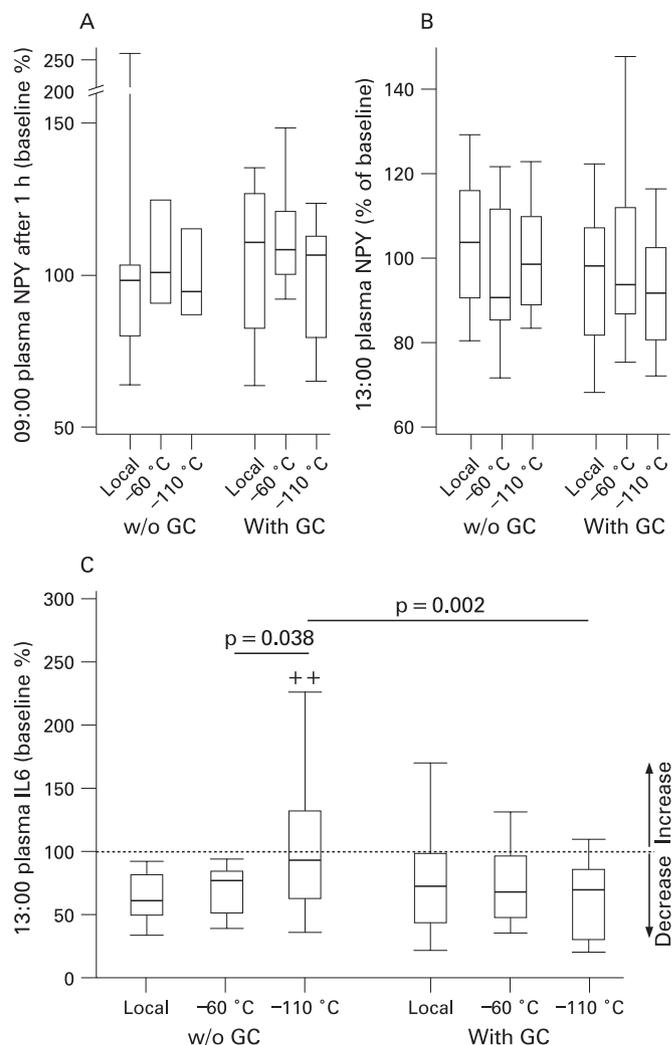


**Figure 3** Change of cortisol relative to dehydroepiandrosterone (DHEA) and 17-hydroxyprogesterone (17OHP) after cold stress. All panels demonstrate the combined data of all 7 days because no significant changes were observed during the entire period (for calculation, see explanation in legend to fig 2A). The 13:00 value is given as percentage of baseline with respect to the ratio of plasma cortisol/plasma DHEA (A) and the ratio of plasma cortisol/plasma 17OHP (B). \* $p < 0.05$ , \*\* $p < 0.005$  vs local cold therapy in the respective treatment group. Box plots show the 10th, 25th, 50th (median), 75th and 90th percentile. w/o GC, without glucocorticoids; with GC, with glucocorticoids.

predominance to cortisol relative to DHEA and 17OHP has been demonstrated in patients with chronic inflammatory diseases (eg, Straub *et al*).<sup>28</sup> In the present study, in patients without GC, the same pathway predominance is visible. Importantly, the pathway predominance is changed in patients under GC therapy, because, now, there is a decrease of cortisol relative to DHEA and 17OHP, particularly in the  $-110^{\circ}\text{C}$  group. This indicates that endogenous cortisol is lost to an even greater degree in steroid-treated patients under cold stress.

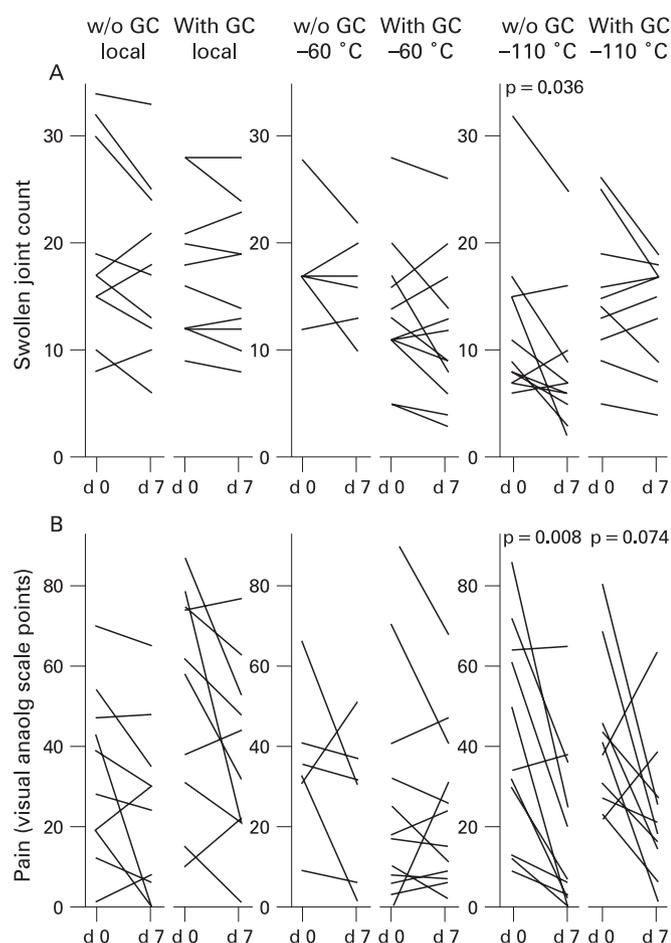
Similarly, the SNS is usually activated using different cold stress paradigms.<sup>16–19</sup> Since NPY is an excellent marker of sympathetic activation,<sup>34–39</sup> NPY should increase after a strong cold stress as applied in this study. However, plasma levels of NPY remained at the 100% level, which means that the post stress value did not increase. Under consideration of a blood half-life for NPY of approximately 30 min<sup>30</sup> and plasma levels independent of the circadian rhythm,<sup>31</sup> one would expect an increase of plasma NPY shortly after a cold stress (1 h after the stress at 9:00 am). These findings are highly suggestive of an inadequate response of the SNS shortly after cold stress. Recently, others have demonstrated a very similar inadequate response with respect to norepinephrine release scleroderma.<sup>14</sup> Our findings corroborate this earlier study in patients with scleroderma, and the results were independent of prior GC therapy. This indicates that not only the HPA axis, but also the SNS, is disturbed.

In a recent study in patients with JIA, a strong cold stressor led to increased lipopolysaccharide-stimulated IL6 secretion from peripheral mononuclear cells.<sup>40</sup> Similarly, patients with RA demonstrated increased IL6 blood levels during psychological stress before surgery.<sup>41</sup> A recent study in patients with RA under psychological stress also demonstrated the increase of stimulated macrophage tumour necrosis factor (TNF), which was abrogated by anti-TNF therapy.<sup>42</sup> Now, we have corroborated



**Figure 4** Changes in neuropeptide Y (NPY) and interleukin 6 (IL6) levels during the morning hours after three sessions of cold stress. All panels demonstrate the combined data of all 7 days because no significant changes were observed during the entire period (for calculation, see explanation in legend to fig 2A). The 13:00 value is given as percentage of baseline with respect to plasma NPY 1 h after the first cold stress session (A), plasma NPY at 13:00 (B) and IL6 at 13:00 (C). \*\* $p < 0.005$  vs local cold therapy in the respective treatment group. Box plots show the 10th, 25th, 50th (median), 75th and 90th percentile. w/o GC, without glucocorticoids; with GC, with glucocorticoids.

that patients with RA without GC demonstrate the highest plasma IL6 levels, which was not observed in patients under GC treatment. Thus, it seems that patients with RA under GC are protected from a cold stress-induced increase of IL6 secretion, possibly due to the expected anti-inflammatory effect of GC. Since IL6 has a strong circadian rhythm with a peak at 07:00 (100%), 13:00 values should be markedly lower at approximately 40%, as recently analysed.<sup>24</sup> From this point of view, the 100% level at 13:00 in this study is suggestive of an activation of IL6 secretion mediated by cold stress. These studies may give the idea that aberrations of stress axes in RA and JIA patients can lead to increased IL6 responses. In this context, it is remarkable and unexpected that the patients under  $-110^{\circ}\text{C}$  cold stress and without GC, those with the highest IL6 serum levels after stress, demonstrated the best clinical response with respect to swollen joint count and pain scores. Thus, the elevated IL6 level is not necessarily linked to an unfavourable outcome. This suggests that



**Figure 5** Clinical responses of individual patients during different cold stress paradigms. Results for the swollen joint count (A) and pain (B) are given. Each line represents one individual patient. The p value indicates a significant difference using the Wilcoxon signed rank test. d, day; w/o GC, without glucocorticoids; with GC, with glucocorticoids.

elevated cortisol levels in the  $-110^{\circ}\text{C}$  stress group without GC is more relevant for the clinical outcome as compared to elevated IL6 levels.

In conclusion, this study demonstrated a relatively inadequate stress response of the HPA axis and, particularly, of the SNS. In patients without GC, cortisol secretion is maintained by a shift of adrenal steroid hormone production from adrenal androgens to cortisol, which is the opposite of what is seen in patients with GC. The inadequate stress response to cold stress was followed by a relative increase of IL6 in patients without GC (particularly in the  $-110^{\circ}\text{C}$  group). This might be an unwanted phenomenon of therapeutic cryotherapy, however these patients demonstrated the best clinical outcome. It seems that the increase of cortisol is more important than the increase of plasma IL6 in determining the clinical outcome. Under consideration of this information, further cryotherapy studies are needed that define further framework conditions for patients with RA in order to generate favourable treatment effects.

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**Competing interests:** None.

**Ethics approval:** The study protocol was approved by the Ethical Committee of Päijät-Häme Hospital district, Finland.

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