Exercise-induced release of cytokines in patients with major depressive disorder

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1. Introduction

During infection, common symptoms are depressed mood, fatigue, problems to concentrate and lack of appetite. This cluster of symptoms is often referred to as "Sickness behaviour", and resembles the symptoms of major depressive disorder (MDD). Therefore, it was originally postulated that there might be a connection between inflammation and depression (Smith, 1991). Since then, this research field has grown and a large amount of studies show elevated immune parameters in patients with MDD as well as other psychiatric disorders (Dantzer et al., 2008).

Patients with MDD frequently, although not invariably, display elevated plasma levels of the pro-inflammatory cytokines interleukin-6 (IL-6), IL-1β and tumour necrosis factor-alpha (TNF-α) (Berk et al., 1997; Thomas et al., 2005; Hestad et al., 2003; Marques-Deak et al., 2007). We have recently shown that depressed patients who attempted suicide had elevated levels of IL-6 in the cerebrospinal fluid...
exercise would also be increased in MDD patients. Such a regulatory or anti-inflammatory substances from muscular tissue would be increased in MDD patients.

In addition, we hypothesized that the production of inflammatory substances in response to contraction (Steensberg et al., 2001). The first cytokine that was discovered being rst cytokine that was discovered being the origin of the elevated cytokines in MDD is still under debate. They may be produced by immune cells in the blood, and indeed some studies show abnormalities in the cytokine production from peripheral monocytes in depressed patients (Lanquillon et al., 2000). Adipose tissue also has the capacity of cytokine production and release to the blood stream (Wozniak et al., 2009). Skeletal muscle is yet another producer of cytokines that could be of importance. Skeletal muscles can be injured during exercise, which may induce inflammation. However, even without injury skeletal muscle produces cytokines in response to contraction (Steensberg et al., 2001). The first cytokine that was discovered being produced by muscles was IL-6. Later it was found that also IL-8 and IL-15 can be produced by contracting muscle tissue in healthy subjects (Chan et al., 2004; Nieman et al., 2003).

The aim of this study was to investigate whether muscular tissue may be responsible for the alterations in cytokine levels that have been observed in patients with MDD. Our primary hypothesis was that production of pro-inflammatory substances from muscular tissue would be increased in MDD patients. In addition, we hypothesized that the production of regulatory or anti-inflammatory substances in response to exercise would also be increased in MDD patients. Such a finding would potentially contribute to a long-term beneficial effect of chronic exercise on depressive symptoms, which has been described (Mead et al., 2009). We therefore subjected medication-free patients and healthy controls to a bicycle challenge, and measured cytokine production throughout the test. Since muscular tissue potentially may produce inflammatory substances with both beneficial and deleterious effects on depressive symptoms, we chose to investigate a total of 15 factors, with pro-, anti-inflammatory and regulatory properties.

### Additional diagnosis

<table>
<thead>
<tr>
<th>Medication</th>
<th>Controls</th>
<th>MDD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contraceptive pills (N = 2)</td>
<td>Contraceptive pills (N = 2)</td>
<td></td>
</tr>
<tr>
<td>Zopiclone (N = 1)</td>
<td>Zopiclone (N = 1)</td>
<td></td>
</tr>
<tr>
<td>Zolpidem (N = 1)</td>
<td>Zolpidem (N = 1)</td>
<td></td>
</tr>
<tr>
<td>Fibromyalgia (N = 1)</td>
<td>Fibromyalgia (N = 1)</td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis (inactive) (N = 1)</td>
<td>Ulcerative colitis (inactive) (N = 1)</td>
<td></td>
</tr>
</tbody>
</table>

### Results

**Exercise test**

The exercise test was performed on a computerized ergometer cycle (Rodby 380, Siemens Elma, Solna, Sweden) in a standardised way (Atterhog et al., 1979). The participants were instructed not to take part in sporting activities on the day of the test or the preceding day, nor eat, drink caffeine-containing beverages or smoke during 2 h preceding the test. The exercise test was designed with an initial workload of 30 W for women and 50 W for men. The workload was increased in small steps (5 W/30 s for women and 5 W/20 s for men) until a heart rate (HR) of 125 ± 5 bpm was attained. The exercise was followed by constant workload, lasting 6 min ('sub-maximal workload'). Subsequently the workload

### Table 1

Demographic data and exercise test-variables of the control and patient groups. There were no significant differences between the groups for age, sex, sub-maximal or maximal work rate, rated perceived exertion or heart rate (NS).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls</th>
<th>MDD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean)</td>
<td>33.7</td>
<td>34.4</td>
</tr>
<tr>
<td>Smoking (yes/no)</td>
<td>0/18</td>
<td>4/14</td>
</tr>
<tr>
<td>Maximal work rate (W)</td>
<td>233 ± 79</td>
<td>203 ± 61</td>
</tr>
<tr>
<td>Heart rate rest (bpm)</td>
<td>66 ± 11</td>
<td>65 ± 14</td>
</tr>
<tr>
<td>Heart rate sub-max (bpm)</td>
<td>127 ± 5</td>
<td>126 ± 4</td>
</tr>
<tr>
<td>Heart rate max (bpm)</td>
<td>186 ± 11</td>
<td>183 ± 12</td>
</tr>
<tr>
<td>Rated perceived exertion (at sub-max work rate)</td>
<td>13.6 ± 1.1</td>
<td>14.0 ± 1.6</td>
</tr>
<tr>
<td>Rated perceived exertion (at max work rate)</td>
<td>18.9 ± 1.6</td>
<td>18.8 ± 1.5</td>
</tr>
<tr>
<td>Medication</td>
<td>Contraceptive pills (N = 2)</td>
<td>Contraceptive pills (N = 2)</td>
</tr>
<tr>
<td>Additional diagnosis</td>
<td>Allergy (N = 2)</td>
<td>Allergy (N = 1)</td>
</tr>
<tr>
<td></td>
<td>Shoulder inflammation (N = 1)</td>
<td>Asthma (N = 1)</td>
</tr>
<tr>
<td></td>
<td>Athletes foot (N = 1)</td>
<td>Migraine (N = 1)</td>
</tr>
</tbody>
</table>

2. Methods

2.1. Subjects

The patients were recruited from the psychiatric clinic at Lund University Hospital. Inclusion criteria was a DSM-IV diagnosis of moderate to severe MDD and scoring of more than 21 on Montgomery Asberg Depression Rating Scale (MADRS). Additional diagnoses and medications taken by the patients are listed in Table 1. Exclusion criteria were pregnancy, cardiovascular disease and treatment with antidepressants, neuroleptics or mood stabilizers during the last month. The control group was randomly selected from the municipal population register in Lund, Sweden. Patients and controls were sex and age matched ± 5 years, and the inclusion criteria for control subjects were good physical health, no history of current mental or somatic disorder. No patient or control had any cardiovascular disorder, diabetes or drug or alcohol abuse.

The research examination of the patients started within a week. The day before the exercise test, patients were evaluated using the Comprehensive Psychopathological Rating Scale (CPRS) (Asberg and Schalling, 1979) including a re-evaluation of MADRS (Svanborg and Asberg, 1994), and Structured Clinical Interview for DSM-IV (SCID-II).

2.2. Exercise test

The exercise test was performed on a computerized ergometer cycle (Rodby 380, Siemens Elma, Solna, Sweden) in a standardised way (Atterhog et al., 1979). The participants were instructed not to take part in sporting activities on the day of the test or the preceding day, nor eat, drink caffeine-containing beverages or smoke during 2 h preceding the test. The exercise test was designed with an initial workload of 30 W for women and 50 W for men. The workload was increased in small steps (5 W/30 s for women and 5 W/20 s for men) until a heart rate (HR) of 125 ± 5 bpm was attained. The exercise was followed by constant workload, lasting 6 min ('sub-maximal workload'). Subsequently the workload
**A**

IL-6 (pg/ml)

Sample nr

--- Controls

Depressed

***

**

* = p < 0.05, ** = p < 0.01, *** = p < 0.001

--- Controls

Depressed

***

**

* = p < 0.05, ** = p < 0.01, *** = p < 0.001

--- Controls

Depressed

***

**

* = p < 0.05, ** = p < 0.01, *** = p < 0.001

--- Controls

Depressed

***

**

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--- Controls

Depressed

***

**

* = p < 0.05, ** = p < 0.01, *** = p < 0.001

--- Controls

Depressed

***

**

* = p < 0.05, ** = p < 0.01, *** = p < 0.001
was increased in small steps (as described above) until exhaustion (‘maximal workload’) (Wisen and Wohlfart, 2004). The ECG (ECGMegachraft, Siemens-Elema, Solna, Sweden) and heart rate were continuously monitored, and the blood pressure (‘BP’) was determined every second minute. Baseline blood samples were taken at 14:00 after 60 min of resting sitting in a chair, right before the exercise test. Blood samples were subsequently taken at sub-maximal and maximal workloads. After the exercise, subjects rested in a supine position and blood samples were drawn after 30 min and 60 min.

2.3. Biochemical analyses

The blood samples were immediately placed on ice and centrifuged at 4 °C and 3000 rpm for 10 min within 1 h of collection. Plasma was stored at −80 °C until analysis. All samples were handled identically. Serum amyloid A (SAA), IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-α, Soluble vascular cell adhesion molecule (S-VCAM), Soluble inter-cellular adhesion molecule (S-ICAM), C reactive protein (CRP) and interferon γ (IFN-γ) were measured in plasma using ultra-sensitive multiplex electrochemiluminescence immunoassays according to the manufacturer’s recommendations (Meso Scale Discovery, UK). All the standards and samples were analysed in duplicates. The sensitivities of the 15 assays are given in Supplementary data.

2.4. Statistical analyses

Measured biological values all displayed a skewness >2 and therefore non-parametric tests were used. To analyse differences between blood samples in the same individual, Friedman’s test was used to screen for significance; followed by Wilcoxon signed rank test. We used (max — baseline) values and (sub-max — baseline) values to calculate increase of the biological factors at the respective timepoints. To analyse differences on a group level, we used Mann–Whitney U-tests or Student’s t-test depending on data distribution. Multiple post-hoc tests were Bonferroni corrected. Analyses were computed using SPSS version 17.0. Alpha-level of significance was set at p<0.05 (two tailed).

2.5. Ethical approval

The study was approved by the Lund University Medical Ethics Committee. Patients signed a written informed consent.

3. Results

3.1. Baseline group-wise comparisons

The MDD patients and healthy controls did not differ with respect to sex, age or to intensity of the workload in the exercise test (Table 1) (Student’s t-test, NS). There was no difference in baseline plasma levels of any of the measured substances between patients and healthy controls (Mann–Whitney U-tests, NS).

3.2. Exercise-induced changes in inflammatory substances

To investigate time-dependent changes in the levels of inflammatory factors during and after the exercise, we compared the intra-individual changes over times using Friedman’s test followed by Wilcoxon Signed Rank. There was a significant increase of cytokines IL-6, IL-8 and TNF-α during the exercise challenge in both depressed patients and healthy controls (Fig. 1A–C). IL-4 levels decreased significantly in both depressed patients and healthy controls during exercise (Fig. 1D). IFN-γ decreased significantly in healthy controls, but not in depressed patients (Fig. 1E).

We next calculated magnitude of increase at sub-max and max timepoints, respectively, of the five factors that changed significantly over time. We found that the decrease in plasma IL-6 was significantly greater in controls than patients at the sub-max timepoint (Kruskal–Wallis ANOVA followed by Mann–Whitney U-test; p = 0.014, Bonferroni correction). There were no other significant differences between the groups in the magnitudes of increase or decrease of the different factors.

4. Discussion

In this study, we measured 15 inflammatory factors in the blood of medication-free MDD patients and healthy controls in response to acute exercise. We found that exercise-induced significant changes over time in cytokine plasma levels in both MDD patients and controls. The levels of IL-8, IL-6 and TNF-α increased throughout the test, and there was a decrease of IL-4 in both groups. In addition, in the healthy controls only there was a significant decrease of IFN-γ. Plasma content of SAA, S-VCAM, S-ICAM, CRP, IL-10, IL-12, IL-13, IL-2, IL-5 or IL-1β neither in MDD patients nor healthy control subjects (Friedman’s test, NS).

Interestingly, the initial decrease of plasma IL-6 was significantly greater in controls than in patients. Both IL-6 and IL-8 have previously described roles in exercise physiology. There are several reports of increased plasma levels of IL-6 in response to acute exercise in healthy humans (Chan et al., 2004; Nielsen and Pedersen, 2008; Akerstrom et al., 2005; Ostrowski et al., 2001). IL-8 is an angiogenic factor which may be of importance when skeletal muscle increases the muscle fibre thickness in response to overload (Baggiolini, 2001). IL-6 has been identified as a regulator of hypertrophic muscle growth (Serrano et al., 2008), and it increases glucose uptake both with and without the presence of insulin (Carey et al., 2006). However, studies on the effects of these cytokines on
brain function and depressive symptoms are sparse. Interestingly, high levels of IL-6 have been associated with depressive symptoms both in humans and animals (Lindqvist et al., 2009; Chourbaji et al., 2006).

Reports including the latest Cochrane study found that exercise improves clinical symptoms of depression (Mead et al., 2009; Blumenthal et al., 2007). Although the biological mechanisms by which exercise reduces depression are yet unclear, several studies have indicated that exercise could affect the clinical symptoms of depression through modulation of cytokine levels in the blood and CNS. For example, animal models have shown reduced expression of cytokine mRNA in several brain regions in response to chronic exercise (Chennouai et al., 2008). In a mouse model of Alzheimer’s disease, exercise decreased brain IL-1β levels, and this was associated with increased cognitive performance (Nicol et al., 2008).

The above findings implicate that, theoretically, exercise in MDD patients would reduce levels of pro-inflammatory factors, which would then be beneficial for depressive symptoms. However, in this study we did not detect any change over time, or group differences, of the classical anti-inflammatory cytokines IL-4, IL-10 and IL-13. In contrast, we here show that the pro-inflammatory cytokines IL-6, TNF-α and IL-8 all increase in depressed patients in response to the test. We found an initial group difference at the sub-max timepoint, when IL-6 decreased significantly in the controls compared to MDD patients. If this mechanism is of importance for the regulation of plasma IL-6 levels remains to be established. The main effect of acute exercise was an increase in IL-6 over time in both groups. These results seem contradictory to the hypothesis that exercise would be beneficial for depressive symptoms through a reduction of pro-inflammatory factors in the blood. However, there are studies suggesting that IL-6 released during exercise may exert regulatory and anti-inflammatory effects (Pedersen et al., 2007). It is also of importance to analyse the effects of chronic exercise on cytokine levels in the central nervous system, which may very well be different than the acute changes in plasma. As for IL-4 and IFN-γ, the plasma levels of these cytokines decreased significantly during exercise. Interestingly, there is evidence that exercise-induced release of cortisol may cause a temporary inhibition of cytokine production from T-cells (Gleeson and Bishop, 2005). This may provide an explanation for the decrease of IL-4 and IFN-γ in response to the exercise we observed here.

In the current study, we chose to include a well-defined group of medication-free, moderately depressed patients. None of the patients suffered from e.g. melancholic depression or suicidal behaviour. Therefore, it is still possible that suicidal, melancholic or other depressive subgroups may display different alterations of cytokine production from muscular tissue in response to exercise. This must be evaluated in future studies of inflammatory mechanisms in these latter groups of depressive patients.

5. Conclusion

Exercise induces significant changes in plasma cytokine levels in MDD patients. There was a significant group difference with respect to IL-6 reactivity at the sub-max timepoint. Long-term studies on the effect of muscular-derived cytokines on depressive symptoms are warranted.

Role of funding source

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Conflict of interest

All authors report no biomedical financial interests or potential conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jad.2010.02.133.

References


Interleukin-6 is elevated in the cerebrospinal fluid of suicide attempters and related to symptom severity. Biol. Psychiatry.


