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Differential Effects of Inflammatory and Psychosocial Stress on Mood, Hypothalamic-Pituitary-Adrenal Axis, and Inflammation in Remitted Depression

Peter Niemegeers, M.D.¹,²; Peter De Boer, Ph.D.¹,³; Glenn J.H. Dumont, Ph.D.¹,⁴; Filip Van Den Eede, M.D., Ph.D.¹,⁵; Erik Fransen, Ph.D.⁶; Stephan J. Claes, M.D., Ph.D.⁷; Manuel Morrens, M.D., Ph.D.¹,²,⁸; Bernard G.C. Sabbe, M.D., Ph.D.¹,²,§

† Both authors contributed equally to this work

1. Collaborative Antwerp Psychiatric Research Institute (CAPRI)
   Faculty of Medicine and Health Sciences
   University of Antwerp
   Universiteitsplein 1
   2610 Antwerp, Belgium

2. University Department of Psychiatry
   Campus Psychiatric Hospital Duffel
   Stationsstraat 22C
   2570 Duffel, Belgium

3. Janssen Research & Development, a division of Janssen Pharmaceutica N.V.
   Turnhoutseweg 30
   2340 Beerse, Belgium

4. Leiden University Medical Centre
   Albinusdreef 2
   2333 ZA Leiden, The Netherlands

5. University Department of Psychiatry
   Campus Antwerp University Hospital
   Wilrijkstraat 10
   2650 Antwerp (Edegem), Belgium

6. StatUa Center for Statistics
   University of Antwerp
   Prinsstraat 13
   2000 Antwerp, Belgium

7. University Psychiatric Centre KU Leuven, campus Leuven
   Herestraat 49
   3000 Leuven, Belgium

8. Psychiatric Hospital Broeders Alexianen
   Provinciesteenweg 408
   2530 Boechout, Belgium

Corresponding author:
Peter Niemegeers
Collaborative Antwerp Psychiatric Research Institute (CAPRI)
Faculty of Medicine and Health Sciences
University of Antwerp – Campus “Drie Eiken”
Universiteitsplein 1
2610 Antwerp, Belgium
E-mail: peter.niemegeers@outlook.com

Contact information of the other authors:
Peter de Boer: pdeboer1@its.jnj.com
Glenn JH Dumont: glenn.dumont@gmail.com
Filip Van Den Eede: filip.van.den.eede@uza.be
Stephan J Claes: stephan.claes@uzleuven.be
Erik Fransen: erik.fransen@uantwerpen.be
Manuel Morrens: manuel.morrens@uantwerpen.be
Bernard GC Sabbe: bernard.sabbe@uantwerpen.be

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Abstract

BACKGROUND/AIMS: Major depressive disorder (MDD) is highly recurrent. This may be due to increased stress sensitivity after remission. Both inflammatory and psychosocial stressors are implicated in the pathogenesis of MDD, but the additive or differential effect is unclear.

METHODS: We conducted a single-blind placebo-controlled study to investigate the effects of inflammatory stress (i.e., typhoid vaccination), psychosocial stress (i.e., Trier Social Stress Test [TSST]), or a combination of both in women (25–45 years old) with (partially) remitted recurrent MDD (n = 21) and healthy female controls (n = 18). We evaluated the effect on mood measured by the Profile of Mood States, markers of the hypothalamic-pituitary-adrenal (HPA) axis activity, and inflammatory system activation. The study was performed during two testing days, separated by a washout of 7–14 days. In a crossover design, subjects received one of the interventions on one day and placebo on the other.

RESULTS: A lowering of mood was seen in patients (β [95% CI] = -4.79 [-6.82 – -2.75], p < 0.001) only after vaccination, but not after the TSST or the combination; this effect was not observed in controls. Controls experienced a significantly different response on Adrenocorticotropic Hormone (ACTH) after vaccination, with a general rise in ACTH not observed in patients. In both groups, the TSST activated the HPA-axis and suppressed the inflammatory parameters.

CONCLUSIONS: There is a differential effect of inflammatory and psychosocial stress on mood and HPA-axis activation in patients with remitted recurrent MDD. This may be an interesting treatment target in MDD.
Main text

1. Introduction

Major depressive disorder (MDD) is a highly recurrent illness and a major cause of disease burden (1). While the exact pathophysiology remains unclear, MDD onset is associated with psychosocial stress (2), as patients experience more negative life events in the year before the first episode (3). Psychosocial stress activates the hypothalamic-pituitary-adrenal (HPA) axis, resulting in glucocorticoid production. Chronic stress and the resulting hyperactivity of the HPA-axis may eventually result in depressive symptoms (4). Accumulating evidence shows that the inflammatory system is also involved in the pathogenesis of depression. Compared to healthy controls, patients with MDD have increased levels of proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (5, 6). Furthermore, treatment of somatic disorders with interferon-α, a proinflammatory cytokine, induces depression in up to 50% of treated patients (7).

At least 60% of patients with MDD will relapse after remission of a single episode (8). While the first episodes of MDD are clearly associated with psychosocial stress, with recurrent episodes, psychosocial stress seems to be progressively less related with episode onset (9). Currently, there are two main theories explaining this phenomenon. The kindling model states that after numerous episodes, depressive episodes become independent of stressors, thereby leading to the spontaneous recurrence of depressive episodes (9). The sensitization model states that the number of depressive episodes is positively associated with sensitization to the effects of stress. Thus, even minor (not identifiable) stressors lead to a relapse, clouding the association between stress and episode onset (3, 10).
Cross-sensitization between different kinds of stressors is possible (11). For example, previous studies in rats showed that an inflammatory challenge potentiates a later response to psychosocial stress (12, 13). Conversely, prior psychosocial stress increased HPA-axis activation and inflammatory response to an inflammatory challenge in rats (14-16). In one study, psychosocial stress increased the negative effects of inflammatory stress in healthy males (17). Compared with healthy controls, women with a history of MDD showed an amplified inflammatory response post-partum, suggesting a sensitized inflammatory response (18).

Although the negative effects of inflammation on mood have been demonstrated in healthy controls (19, 20), to our knowledge, no study has examined the effects of an inflammatory stressor on current or remitted MDD. In the present study, we evaluated the effects of inflammatory and psychosocial stress, and the combination thereof, on mood, inflammation biomarkers, and the HPA-axis, in remitted MDD patients and healthy controls. We hypothesized that MDD patients, even when remitted, show an increased sensitivity to these stressors, indicating a vulnerability factor. We also hypothesized that there would be a cross-sensitization between inflammation and psychosocial stress, with both having additive or synergistic effects. The *Salmonella typhi* vaccination was used as the inflammatory stressor, as it has been shown to induce a transient decrease in mood (19, 20); the “Trier Social Stress Test” (TSST) was used as the psychosocial stressor (21). The first objective was to evaluate the effects of the interventions on mood, measured using the Profile of Mood States (POMS) questionnaire. Secondly, we explored the differential effects of these stressors on several biomarkers of inflammation and HPA-axis functioning.
2. Methods and Materials

2.1 Participants

Twenty-one women with (partially) remitted and recurrent MDD and 18 female controls aged 25–45 years provided informed consent and participated in the study. Three patients dropped out during the study and were replaced. Only women were selected because of possible sex differences in stress and inflammatory response (22). The patient group had moderate to severe recurrent MDD (without psychotic features), currently in (partial) remission, using the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revised (DSM-IV-TR) (8), with the most recent depressive episode being within the last 24 months and a minimum 3-month stable period. Patients had to have a score below 15 on the Montgomery-Åsberg Depression Rating Scale (MADRS) (23), a body mass index (BMI) of 18–30 kg/m², and be medically stable with respect to vital signs, clinical examination, and clinical laboratory tests (blood and urine sample). Exclusion criteria were another axis 1 diagnosis; substance abuse or dependence within the past 6 months (excluding nicotine and caffeine); acute suicidal behavior; a history of serious disease; prior exposure to the TSST; exposure to severe psychosocial stress within the previous 6 months; or typhoid vaccination within the previous 5 years. Participants treated with more than one antidepressant or drugs compromising the immune system were excluded. Controls were recruited using advertisements. Patients were recruited from the participating institutions and the surrounding private practices of psychiatrists, psychologists, and general practitioners.

The study was conducted at the University Department of Psychiatry, campus Psychiatric Hospital Duffel, Belgium, and the University Psychiatric Center KU Leuven, campus Leuven, Belgium. Approval for the study was obtained from the central and local Ethics Committees,
and the Belgian Health Authorities. This study complied with the regulations of the participating institutions, the International Conference on Harmonization Good Clinical Practice guidelines, and the European Directive 2001/20/EC. It was registered under the identifiers NCT01533285 (ClinicalTrials.gov-database) and 2011-004898-80 (EudraCT-database)

2.2 Study Design

We conducted a two-way, randomized, single blind, placebo-controlled crossover study to assess the effects of an inflammatory stressor, a psychosocial stressor, and the combination thereof on mood, cytokine levels, and HPA-axis markers. The first visit consisted of an eligibility screening and also administration of the Childhood Trauma Questionnaire (CTQ), a retrospective self-report questionnaire of childhood abuse and neglect (24). Eligible participants were randomized by the sponsor to one of the treatment sequences using a computer-generated sequence. Only participants were blinded. The study consisted of two treatment periods separated with a washout of 7–14 days, and a follow-up telephone call 7–14 days after the last treatment period, to inform about possible late side effects of the intervention (Figure 1). There were six possible treatment sequences: for each treatment group a sequence with placebo on the first period and active treatment on the second period and vice versa).

At the start of each treatment day, an alcohol breath test and urine drug and pregnancy screening were administered. If applicable, the TSST was administered at 12:00 PM. The TSST is a social stress paradigm in which the participant is asked to do a five-minute fictitious job interview after a three-minute preparation and a five-minute arithmetic exercise
for a cold and distant audience, while speaking into a microphone and being video recorded (21).

At 12:20 PM, the subject received either a placebo injection (0.5 mL NaCl 0.9%) or the typhoid vaccine (0.5 mL containing 25 µg *Salmonella* Typhi capsular polysaccharide; Typhim® Vi, Sanofi Pasteur MSD, Diegem, Belgium). Both the placebo and typhoid vaccine were transferred to similar looking syringes.

Throughout the testing periods, vital signs were monitored regularly. The participant left the study center after the last post-dose measurements.

### 2.3 Assessments

#### 2.3.1 Mood

Mood was measured with the POMS (brief form), which was administered at baseline and 60, 90, 150, 180, 240, and 360 minutes post-dose. The POMS is a self-report questionnaire that measures current mood, yielding scores for total mood and six subscales: activity/vigor, anger/hostility, confusion/bewilderment, depression/dejection, fatigue/inertia, and tension/anxiety (25).

#### 2.3.2 Biological Measures

Serum samples were taken to measure levels of inflammatory cytokines (i.e., interferon-γ [IFN-γ], tumor necrosis factor-α [TNF-α], and interleukin-6 [IL-6]), as well as markers of HPA-axis activation (i.e., adrenocorticotropic hormone [ACTH] and cortisol). Blood samples were taken through an indwelling catheter immediately before the start of the treatment (11:55 am), immediately post-treatment (12:21 pm), and 30, 60, 90, 150, 180, 240 and 360
minutes post-treatment. At 15 minutes post-treatment, an extra cortisol and ACTH sample was taken.

HPA-axis markers were analyzed at PRA International, Zuidlaren, the Netherlands. ACTH and Cortisol were analyzed using a Siemens® IMMULITE 2000 Immunoassay System. Inflammatory markers were analyzed at Janssen Biobank, Beerse, Belgium. The cytokine assays were quantitative electrochemiluminescence immunoassays, performed with Meso Scale Discovery® V-PLEX Proinflammatory Panel 1 (human) kits. The detection ranges were as follows: ACTH, 1.1 to 278 pmol/L; cortisol, 28 to 1380 nmol/L; IFN-γ, 0.2–0.9 to 1060–1320 ng/L; TNF-α, 0.06–0.3 to 320–352 ng/L; and IL-6, 0.07–0.3 to 743–833 ng/L.

2.4 Statistics

Baseline differences between the groups were examined using an unpaired Student’s t-test or Wilcoxon-Mann-Whitney-test for non-normal data. For the mood and biological data, the mean pre-dose score was used to measure baseline differences. For the biological markers, values below quantifiable levels were replaced with the lowest quantifiable concentration. Non-continuous variables were tested with the chi-square test.

The effect of the intervention on the different outcomes was estimated using a linear mixed model. All available data from non-completers was included. As the biological data and the data of several POMS subscales (namely, anger/hostility, depression/dejection, and tension/anxiety) was not normal, a log-transformation was applied. The mixed model included as fixed effects the group (control group or patient), intervention (placebo, typhoid vaccine without TSST, placebo with TSST, or the typhoid vaccine with TSST), time point, and the appropriate interactions. Pre-dose score/concentration, treatment period, study center,
body mass index, age, CTQ, pre-dose MADRS score, and antidepressant use were also included as fixed effects; subject was included as a random effect. When a significant interaction was found, between-group comparisons were performed (e.g., between placebo and the three different treatments), using Bonferroni-correction for multiple testing. The estimated differences ($\beta$) between placebo and the intervention are reported with the 95% confidence interval (CI).
3. Results

An overview of baseline characteristics is summarized in Table 1. While there were no significant differences in demographics and biomarkers between patients and controls, patients had higher scores on the MADRS and the CTQ, as well as a lower POMS score. Fourteen patients (66.7%) were concomitantly treated with an antidepressant. The use of concomitant medication can be found in Table S1 of the online supplement. An overview of the participant flow can be found in Figure 2. A graphical representation of the effects of the interventions on each time point and group is provided in Figure S1-S12 of the online supplement.

3.1 Mood

3.1.1 Total score on the POMS (Figure 3 and Figure S1)

Patients reacted significantly differently on the interventions than controls, as the group × intervention interaction was significant (p < 0.001). A significant general lowering of mood was found in patients after the vaccine without TSST (β [95% CI] = -4.79 [-6.82 – -2.75]; p < 0.001). There were no significant effects on mood of the other two interventions in patients, neither were there of any of the interventions in controls.

3.1.2 Subscales of the POMS

3.1.2.1 Activity/Vigor (Figure S2)

A significant group × intervention interaction was observed (p = 0.010), meaning that patients reacted significantly different than controls. There was a lowering in vigor/activity in patients after the vaccine without TSST (β [95% CI] = -1.55 [-2.50 – -0.60]; p =0.008). There was no
effect of the other interventions in patients, neither was there an effect of any of the interventions in controls.

3.1.2.2 Anger/Hostility (Figure S3)

Patients reacted significantly different on the interventions (group × intervention interaction: p = 0.009). A lowering of anger/hostility was seen in patients after placebo and TSST (β [95% CI] = -0.05 [-0.52 – -0.09]; p =0.008). The other interventions had no effect.

3.1.2.3 Confusion/Bewilderment (Figure S4)

The groups did not react differently on the interventions. A significant effect of intervention was found (p < 0.001). An increase in confusion/bewilderment was observed after placebo and TSST (β [95% CI] = 0.47 [0.16 – 0.77]; p = 0.008), while there was a decrease after vaccine and TSST (β [95% CI] = -0.39 [-0.70 – -0.09]; p = 0.036). No effect was observed after vaccine without TSST).

3.1.2.4 Depression/Dejection (Figure S5)

A significant effect of intervention was found (p = 0.023). The both groups did not differ significantly in their reactions on the interventions. While examination of the single intervention did not show any significant effects of the interventions, there was a trend for an increase in depression/dejection after the vaccine and TSST (β [95% CI] = -0.15 [-0.27 – -0.03]; p = 0.055).

3.1.2.5 Fatigue/Inertia (Figure S6)

Patients reacted significantly different on the intervention compared to controls (p < 0.001). While an increase in fatigue/inertia was seen in patients after vaccine without TSST (β [95%
CI] = 0.91 [0.10 – 1.71]), this was not significant after multiple testing correction. On the other hand, in controls, a significant increase in fatigue/inertia was seen after placebo and TSST (β [95% CI] = 1.41 [0.62 – 2.21]; p = 0.003).

3.1.2.6 Tension/Anxiety (Figure S7)

The groups reacted significantly different, with a significant group × intervention interaction (p = 0.008). In patients, an increase in tension/anxiety was seen after the vaccine without TSST (β [95% CI] = 0.40 [0.10 – 0.73]; p = 0.039). The other interventions had no effect in patients. In controls, an increase was seen after the vaccine with TSST (β [95% CI] = 0.33 [0.11 – 0.58]; p = 0.011), but no effects were seen after the other treatments.

3.2 Biological Measures

3.2.1 Measures of the HPA-axis

3.2.1.1 ACTH (Figure S8)

A significant group × intervention interaction was found (p < 0.001), suggesting that patients reacted differently on the interventions than controls. There was a trend for a general rise of ACTH after vaccine without TSST in controls (β [95% CI] = 0.27 [0.06 – 0.49]; p = 0.056), but not in the patients.

There was a significant time point × intervention interaction (p < 0.001), though no significant group × time point × intervention interaction, meaning that the groups did not differ significantly in response. A significant rise of ACTH occurred immediately after the TSST in both populations, both in the placebo and TSST group (β [95% CI] = 1.00 [0.53 – 1.54]; p < 0.001) and the vaccine with TSST group (β [95% CI] = 0.79 [0.33 – 1.31]; p = 0.012).
In conclusion, a trend for a general rise of ACTH was found in controls after the typhoid vaccine without TSST, but not in patients, in which the two groups differed significantly. In contrast, both groups reacted similarly after the TSST (with placebo and with the typhoid vaccine), with a rise of ACTH immediately after the TSST.

### 3.2.1.2 Cortisol (*Figure S9*)

Both groups reacted similarly on the interventions, as there were no group interactions. A significant time point × intervention interaction was found (p < 0.001), with a rise immediately after (β [95% CI] = 136.4 [69.1 – 216.3]; p < 0.001) and 15 min (β [95% CI] = 120.4 [56.1 – 196.7]; p = 0.001) after placebo and TSST. This effect was however not found after the TSST in combination with the typhoid vaccine.

### 3.2.2 Cytokines

#### 3.2.2.1 IFN-γ (*Figure S10*)

Patients and controls reacted significantly differently on the intervention (group × intervention: p = 0.028). The placebo and TSST intervention lead to a decrease in IFN-γ in both groups (patients: β [95% CI] = -0.79 [-1.04 – -0.52]; p < 0.001; controls: β [95% CI] = -0.66 [-0.91 – -0.39]; p < 0.001). However, in patients there was also a significant increase after the typhoid vaccine and TSST combination (β [95% CI] = 0.84 [0.53 – 1.18]; p < 0.001).

The time point × intervention interaction was also significant (p < 0.001). As there was no group × time point × intervention the analysis applies to both groups. After placebo and TSST, there was a decrease in IFN-γ at 180 minutes (β [95% CI] = -0.76 [-1.16 – -0.35]; p = 0.011), 240 minutes (β [95% CI] = -1.06 [-1.42 – -0.67]; p < 0.001), and 360 minutes (β [95% CI] = -1.33 [-1.75 – -0.89]; p < 0.001).
CI] = -1.48 [-1.83 – -1.10]; p < 0.001). On the other hand, after the combination treatment, an increase was seen at 240 minutes (β [95% CI] = 0.96 [0.46 – 1.51]; p = 0.002) and 360 minutes (β [95% CI] = 0.98 [0.45 – 1.55]; p = 0.004).

In conclusion, in both groups there was a lowering of IFN-γ after placebo and TSST. This lowering was more pronounced from 180 minutes post-intervention onward. The effect of the typhoid vaccine with the TSST on IFN-γ is conflicting: while there seems to be a general rise in patients compared to controls after the intervention, there also is a rise in both groups from 240 minutes post-intervention onward.

3.2.2.2 TNF-α (Figure S11)

No significant differences between the both groups were found. A significant effect of intervention was found (p = 0.011), showing a decrease in TNF-α after placebo and TSST (β [95% CI] = -0.06 [-0.02 – -0.09]; p = 0.007). The other interventions had no effect on TNF-α levels.

3.2.2.3 IL-6 (Figure S12)

No significant effects of the intervention were found. Interestingly, while on baseline no significant difference was found between both groups, there was a significant effect of group, suggesting an increased concentration of IL-6 in patients (β [95% CI] = 0.78 [0.14 – 1.62]; p = 0.015).
4. Discussion

This is the first study to examine the effect of inflammatory stress in patients in remitted MDD, as well as the first study to examine the differential effects of both an inflammatory and a psychosocial stressor in this population. We hypothesized that patients would be more sensitive to the negative effects of inflammatory and psychosocial stress mood and that the combination of both would have an additive or synergistic effect.

In patients, inflammation, but not psychosocial stress, had negative effects on mood. This suggests that there is indeed an increased sensitivity for the effects of inflammatory stress on mood in remitted MDD patients. Examination of the subscales reveals that this mood decrease was partly due to a decrease in vigor/activity and a non-significant increase in fatigue/inertia. There was also an increase in tension/anxiety, but no change in the depression/dejection subscale. The changes in vigor/activity and fatigue/inertia suggest that patients were more sensitive to the development of sickness behavior following inflammatory stress. Previous research indeed showed that increased inflammation is particularly associated with the somatic symptoms of depression and anxiety (26, 27). This can explain why the inflammatory stressor affected only activity, fatigue, and anxiety, but not depressive mood. However, research on mice showed that sickness behavior following inflammatory stress was an early effect, while depressive symptoms followed later (28). A similar pattern is seen in patients treated with interferon, where an increase in neurovegetative symptoms (such as fatigue and psychomotor impairment) is an early side effect of the treatment, while depressive symptoms usually occur later in treatment (7). This early increase in neurovegetative symptoms was also shown to predict cognitive depressive symptoms later in therapy (29).
No negative effects were observed of the TSST, as would be expected (30). However, it should be noted that in previous studies, mood was measured directly after TSST, while in this study mood was only first measured 60 minutes post-intervention. Examination of the subscales of the mood scale did reveal some changes after the TSST (increased confusion/bewilderment in both groups, decreased anger/hostility in patients, and increased fatigue/inertia in controls), which don’t seem to follow a clear pattern.

The TSST activated the HPA-axis in both groups, further validating it as a paradigm for induction of HPA-axis activation. The TSST decreased inflammatory cytokines, probably due to cortisol’s anti-inflammatory effect. While this anti-inflammatory effect may seem positive, it should be noted that the onset of MDD is associated more with chronic stress, while the TSST is a short and relatively mild stressor. Prolonged psychosocial stress and the associated HPA-axis hyperactivation lead to glucocorticoid receptor desensitization and glucocorticoid resistance, culminating in reduced anti-inflammatory effects (31-33). However, recent evidence shows that glucocorticoids are not exclusively anti-inflammatory, and can, in certain contexts, enhance pro-inflammatory responses, possibly through microglia activation, which is associated with psychiatric disorders including major depressive disorder (34, 35).

Pro-inflammatory states are also known to activate the HPA-axis, which acts as a negative feedback loop controlling the immune response (36, 37). This expected response was observed in controls following vaccination, but was absent in patients. This may be due to HPA-axis dysfunction (reduced activation of the HPA-axis in the current study) in the patient group, which has been shown to persist after remission in a proportion of the patients with recurrent and remitted MDD (38, 39). However, we did not observe any significant differences in baseline HPA-axis activity between patients and control subjects, and both
groups reacted similarly on the TSST. This may implicate an immune-specific reduced activation of the HPA-axis after inflammatory stress in remitted MDD, but this finding requires further investigation and replication in future studies.

While the vaccine without TSST did reduce mood in patients, the combined intervention (vaccine and TSST) did not. On the subscales of the mood scale, only a non-significant increase in depression/dejection was found and in controls an increase in tension/anxiety and a decrease in confusion/bewilderment. It can be hypothesized that the anti-inflammatory effect of the TSST negated the pro-inflammatory effect of the vaccine, though this cannot be clearly seen in the biomarker data of the combination intervention. An increase in IFN-γ was seen in patients only after combination treatment, but not after the single treatments. This indicates a synergistic effect of both stressors in patients, reflecting an earlier cross-sensitization, as we initially hypothesized. This difference was however not reflected in the behavioral measures. However, our study may have been underpowered to find small differences. Alternatively, it can be hypothesized that the mood-altering effects of these increases in cytokine levels appeared after the observation period of 6 hours.

This increased sensitivity to inflammation may be a trait that may increase MDD risk. In the present study, no effect of inflammatory stress on mood was found in controls; however, other studies (17, 19, 20, 40-43), although not all (44), observed negative effects of inflammation in healthy controls. Interestingly, personality traits such as optimism protect against inflammation-induced mood decreases (45). This indicates a subgroup in the general population that is more sensitive to inflammation. Indeed, several risk factors for increased pro-inflammatory states are described in the literature, such as childhood maltreatment (46,
which is also associated with increased stress responsivity and depression (31). However, our analysis controlled for these factors.

Strengths of the study are the studied population and the design. Most research is focused on patients during a major depressive episode. Selection of a population without active disease made it possible to observe if any disturbances persist after remission. The study was also designed to measure two different kinds of stressors, examining several disease facets. This is, to our knowledge, the first study to measure both inflammatory and psychosocial stress in a clinical population. However, some limitations have to be mentioned. First, the sample size was relatively small. Secondly, the cross-sectional design does not allow an examination of causality (predisposing factor in MDD or biological scar after recurrent depression). Third, the majority of patients took antidepressants, which can normalize HPA-axis disturbances and pro-inflammatory states (32, 48). Finally, the effects of the interventions were examined within a short time frame (6 h), although there may be additional and larger long-term effects.

In conclusion, patients with recurrent and remitted MDD were sensitive to the mood-lowering effects of inflammatory stress, contrary to controls. Moreover, the HPA-axis was not activated after inflammation in patients. Further research is needed to determine whether this is due to a trait that increases the risk for MDD or due to the consequences of multiple major depressive episodes. However, there was no increased sensitivity to psychosocial stress, suggesting that different types of stressors have different effects. While no synergistic effects of both stressors on mood were observed, the combination of both stressors had synergistic effects on inflammatory markers in the patient group. Overall, these findings corroborate previous reports that inflammatory processes are involved in MDD. In the present study, disturbances in the inflammatory system appear to persist after remission, which may play a
role in recurrence. This increased sensitivity to inflammatory stress may be an interesting
treatment target for the prevention of MDD recurrence.
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Table 1: Baseline demographic and clinical characteristics

<table>
<thead>
<tr>
<th>Measure</th>
<th>Patients (n = 21)</th>
<th>Controls (n = 18)</th>
<th>p-value</th>
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<tr>
<td>Age</td>
<td>33.9 (7.02)</td>
<td>32.7 (6.65)</td>
<td>n.s.</td>
</tr>
<tr>
<td>BMI</td>
<td>24.0 (2.80)</td>
<td>22.4 (3.18)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Education years</td>
<td>15.2 (2.40)</td>
<td>15.7 (2.97)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Right-handedness (%)</td>
<td>85.0%</td>
<td>82.4%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Antidepressant use (%)</td>
<td>66.7%</td>
<td>0.0%</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>European descent</td>
<td>95.2%</td>
<td>88.9%</td>
<td></td>
</tr>
<tr>
<td>Maghrebi descent</td>
<td>0.0%</td>
<td>11.1%</td>
<td></td>
</tr>
<tr>
<td>African descent</td>
<td>4.8%</td>
<td>0.0%</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MADRS</td>
<td>6.38 (5.29)</td>
<td>0.64 (1.07)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>CTQ</td>
<td>42.7 (16.66)</td>
<td>31.4 (6.58)</td>
<td>.008</td>
</tr>
<tr>
<td>POMS</td>
<td>-14.60 (17.34)</td>
<td>0.92 (7.60)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td><strong>Biomarkers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPA-axis measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>295 (132)</td>
<td>313 (146)</td>
<td>n.s.</td>
</tr>
<tr>
<td>ACTH (pmol/L)</td>
<td>2.58 (1.924)</td>
<td>2.35 (1.586)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Inflammation measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ (ng/L)</td>
<td>6.63 (6.40)</td>
<td>7.34 (13.60)</td>
<td>n.s.</td>
</tr>
<tr>
<td>TNF-α (ng/L)</td>
<td>1.81 (1.46)</td>
<td>1.48 (0.37)</td>
<td>n.s.</td>
</tr>
<tr>
<td>IL-6 (ng/L)</td>
<td>0.76 (0.49)</td>
<td>0.68 (0.38)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

ACTH: Adrenocorticotropic hormone; BMI: body mass index; CTQ: Childhood Trauma Questionnaire; IFN-γ: Interferon-γ; IL-6: Interleukin-6; MADRS: Montgomery-Åsberg Depression Rating Scale; n.s.: not significant; POMS: Profile of Mood States; TNF-α: tumor necrosis factor-α
Figure 1: Study design

- **Screening** (Day -21 to -1)

  - **Period 1** (placebo or active intervention) 7–14 days

  - **Period 2** (placebo or active intervention) 7–14 days

  - **Follow-up telephone call**
Figure 2: Participant flow

**Patients:**
assessed for eligibility: n = 31

- Excluded: n = 10
did not meet inclusion criteria/met exclusion criteria.

- Randomized: n = 21
  - Typhoid vaccine: n = 8
  - Placebo and TSST: n = 7
  - Typhoid vaccine and TSST: n = 6

  - completed the study: n = 18
  - discontinued after period 1: n = 3 (reasons: recurrence of MDD, pregnancy, and difficulty getting blood samples)
  - none lost to follow-up

- Analyzed: n = 21

**Controls:**
assessed for eligibility (n = 21)

- Excluded: n = 3
did not meet inclusion criteria/met exclusion criteria.

- Randomized: n = 18
  - Typhoid vaccine: n = 6
  - Placebo and TSST: n = 6
  - Typhoid vaccine and TSST: n = 6

  - all completed the study
  - no drop-outs
  - none lost to follow-up

- Analyzed: n = 18

TSST: Trier Social Stress Test
Figure 3: Least square means of the general Profile of Mood States score after each intervention

*: p < 0.05; TSST: Trier Social Stress Test
Differential Effects of Inflammatory and Psychosocial Stress on Mood. Hypothalamic-Pituitary-Adrenal Axis. and Inflammation in Remitted Depression

Online Supplement

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## Table S1: Concomitant medication use

<table>
<thead>
<tr>
<th>Medication</th>
<th>Patients (n = 21)</th>
<th>Controls (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alimentary Tract</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loperamide</td>
<td>0%</td>
<td>5.56%</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Analgesics and Anti-Infectives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic Acid *</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Paracetamol **</td>
<td>4.76%</td>
<td>16.67%</td>
</tr>
<tr>
<td>Roxithromycin *</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Blood and blood forming organs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enoxaparin sodium</td>
<td>0%</td>
<td>5.56%</td>
</tr>
<tr>
<td><strong>Dermatological</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcipotriol/Betamethasone Dipropropionate (topical)</td>
<td>0%</td>
<td>5.56%</td>
</tr>
<tr>
<td>Isotretinoin</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Ketoconazole (topical)</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Terbinafine (topical)</td>
<td>0%</td>
<td>5.56%</td>
</tr>
<tr>
<td><strong>Anticonception</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyproterone Acetate</td>
<td>0%</td>
<td>5.56%</td>
</tr>
<tr>
<td>Cyproterone Acetate/Ethinylestradiol</td>
<td>4.76%</td>
<td>11.11%</td>
</tr>
<tr>
<td>Desogestrel/Ethinylestradiol</td>
<td>9.52%</td>
<td>16.67%</td>
</tr>
<tr>
<td>Drospirenone/Ethinylestradiol</td>
<td>0%</td>
<td>11.11%</td>
</tr>
<tr>
<td>Estradiol/Nomegestrol Acetate</td>
<td>0%</td>
<td>5.56%</td>
</tr>
<tr>
<td>Ethinylestradiol</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Ethinylestradiol/Etonogestrel (vaginal contraceptive ring)</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Ethinylestradiol/Gestodene</td>
<td>4.76%</td>
<td>5.56%</td>
</tr>
<tr>
<td>Ethinylestradiol/Norgestimate</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Levonorgestrel</td>
<td>14.29%</td>
<td>16.67%</td>
</tr>
<tr>
<td>Levonorgestrel (intrauterine contraceptive device)</td>
<td>0%</td>
<td>5.56%</td>
</tr>
<tr>
<td><strong>Nervous System</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alprazolam</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Duloxetine</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Escitalopram</td>
<td>14.29%</td>
<td>0%</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Flurazepam</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Nortriptyline</td>
<td>4.76%</td>
<td>0%</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>9.52%</td>
<td>0%</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>14.29%</td>
<td>0%</td>
</tr>
<tr>
<td>Sertraline</td>
<td>9.52%</td>
<td>0%</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>19.05%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Vitamins, Minerals, and Food supplements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B containing supplements</td>
<td>14.29%</td>
<td>5.56%</td>
</tr>
<tr>
<td>Iron containing supplements</td>
<td>9.52%</td>
<td>11.11%</td>
</tr>
<tr>
<td>Unspecified vitamins and minerals</td>
<td>4.76%</td>
<td>5.56%</td>
</tr>
<tr>
<td>Unspecified homeopathy and other food supplements</td>
<td>4.76%</td>
<td>5.56%</td>
</tr>
</tbody>
</table>

* This medication was used at screening but was discontinued before the start of the study.
** The occasional intake of paracetamol was permitted throughout the study provided no paracetamol was taken within 48 hours prior to the intervention.
2 Results of the interventions on the Profile of Mood States (POMS)

2.1 Figure S1: POMS Total Score

The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

*: p < 0.05 (on scores in general); TSST: Trier Social Stress Test
2.2 Figure S2: POMS Activity/Vigor Subscale

The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

*: p < 0.05 (on scores in general); TSST: Trier Social Stress Test
2.3 Figure S3: POMS Anger/Hostility Subscale

The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

*: p < 0.05 (on scores in general); TSST: Trier Social Stress Test
2.4 **Figure S4: POMS Confusion/Bewilderment Subscale**

The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

*: p < 0.05 (on scores in general); TSST: Trier Social Stress Test
2.5 Figure S5: POMS Depression/Dejection Subscale

The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

°: p < 0.1 (on scores in general); TSST: Trier Social Stress Test
2.6 Figure S6: POMS Fatigue/Inertia Subscale

The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

*: p < 0.05 (on scores in general); TSST: Trier Social Stress Test
2.7 **Figure S7: POMS Tension/Anxiety Subscale**

The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

*: p < 0.05 (on scores in general); TSST: Trier Social Stress Test
3 Results of the interventions on the biological measures

3.1 Figure S8: Adrenocorticotropic Hormone (ACTH)

The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

°: p < 0.1 (on blood concentration in general); +: p < 0.05 (on specific time points); TSST: Trier Social Stress Test
3.2 Figure S9: Cortisol

The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

+: p < 0.05 (on specific time points); TSST: Trier Social Stress Test
The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

*: p < 0.05 (on blood concentration in general); †: p < 0.05 (on specific time points);
TSST: Trier Social Stress Test
**3.4 Figure S11: Tumor Necrosis Factor-α**

The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

*: p < 0.05 (on blood concentration in general); TSST: Trier Social Stress Test
The least squares mean concentration with 95% confidence intervals are shown in this figure. Pre-dose concentration (mean concentration with 95% confidence interval of the whole group at 20 m before intervention) is also included.

TSST: Trier Social Stress Test