Mindfulness as a Predictor of IL-6 Response to a Repeated Stressor

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ABSTRACT

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A thesis presented to the Psychology Department

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We examined the hypothesis that mindfulness is associated with lower levels of stress-induced inflammation. On two consecutive days, plasma concentrations of interleukin-6 (IL-6) were assessed at baseline and at 30 and 120 min following exposure to a standardized laboratory stressor in a sample of 46 healthy young adults. Participants who were higher in trait mindfulness exhibited significantly lower IL-6 responses to a novel stressor, even when controlling for subjective distress, age, gender, and BMI. Mindfulness was not related to IL-6 response to a repeated stressor. These findings suggest that mindfulness may serve as a protective factor against stress-induced inflammation in response to novel stressors.
TABLE OF CONTENTS

I. List of Tables……………………………………………………………………………….vi
II. List of Figures………………………………………………………………………………vii
III. Introduction……………………………………………………………………………1
IV. Method……………………………………………………………………………….3
V. Results………………………………………………………………………………….7
VI. Discussion……………………………………………………………………………..8
VII. Appendix A: Tables………………………………………………………………..11
VIII. Appendix B: Figures………………………………………………………………14
IX. References…………………………………………………………………………..18
LIST OF TABLES

1. Descriptive statistics of TSST1 and TSST2 IL-6 levels

2. Descriptive statistics of Mindfulness, BMI, TSST1 Distress and TSST2 Distress
LIST OF FIGURES

1. Mean IL-6 levels at baseline and 30 and 120 min post-TSST on days 1 and 2

2. Mindfulness predicting TSST1 IL-6 response

3. Mean TSST 1 IL-6 levels at baseline and 30 and 120 min post-TSST for participants high and low mindfulness (median split).
Mindfulness as a predictor of IL-6 response to a repeated stressor

Previous research shows that stress is pervasive and can harm health, in part via dysregulation of the HPA axis (McEwen, 1998). The immune and neuroendocrine systems are modulated by stress and are relevant to disease development (Carlson, Speca, Patel & Faris, 2007; Taylor, 1995). Social evaluative stress impacts health-relevant biological responses, including hypothalamic-pituitary-adrenal (HPA) axis activation (Dickerson & Kemeny, 2004). To the extent that people confront repeated stressors in daily life, they may face health risks as a result. Eighty percent of workers feel stress on the job and nearly half say that they need help in learning how to manage stress (Murphy & Schoenborn, 1993).

It has also been demonstrated that stress can cause an inflammatory response similar to one from injury or illness but lower in magnitude (Steptoe, Hamer & Chida, 2007). Chronic low-grade inflammation can significantly increase the risk of disease of in a number of diseases, such as cardiovascular disease, cancer, and Alzheimer’s disease (Danesh, Kaptoge, Mann, Sarwar, Angleman & Gudnason, 2008).

Individuals differ in their biological responses to stress, however. Some individuals demonstrate heightened biological reactivity, whereas others show more attenuated responses. One individual difference that may be especially likely to play a role in shaping people’s biological responses to stress is mindfulness. Mindfulness refers to self-regulation of attention to all experiences (Bishop, Lau, Shapiro, Carlson, Anderson, Carmody & Devins, 2004). Individuals who are high in trait mindfulness demonstrate better psychological health (Keng,
Smoski & Robins, 2011). The link between mindfulness and health may be explained in part by healthier biological responses to stress among mindful individuals.

Specifically, mindful individuals may perceive psychosocial stressors as less threatening than those lower in mindfulness, and this decreased threat perception may in turn lead to attenuated biological responses. Supporting this perspective, mindfulness has been shown to decrease perceived stress levels (Haines, Spadaro, Choi, Hoffman & Blazeck, 2014). Social self preservation theory (Dickerson, Gruenewald, & Kemeny, 2004) states that psychosocial stress induces increased peripheral inflammation, as well as other biological responses, when there is a threat to the self in a social evaluative context. Numerous studies provide evidence for the link between social evaluative threat and an increased peripheral inflammation response including pro-inflammatory cytokine activity e.g., (Dickerson, Gruenewald & Kemeny, 2004; Dickerson & Kemeny, 2004). Dickerson and colleagues (Dickerson, Gruenewald & Kemeny, 2004) introduced a stressor characterized by social-evaluative in which the self was judged negatively by a panel of judges and found that this task elicited increased pro-inflammatory cytokine activity.

This study examined the hypothesis that individuals high in trait mindfulness would show a lower inflammation response to a repeated social evaluative stressor compared to those low in trait mindfulness. Mindfulness may regulate the response to a social evaluative stressor because it can serve as a tool that helps reduce the emotional impact of a stressor. Prior studies have shown that mindfulness reduces IL-6 response to a single novel stressor (Tomfohr, Pung, Mills & Edwards, 2015) but none have examined mindfulness as a predictor of IL-6 response with
repeated stressors. Examining responses to repeated stressors is important because the stress people experience in everyday life is often repeated.

**Method**

**Participants**

Data for this research were collected as part of a larger research project conducted over four years in which young adults (age 18-35) and older adults (50-65) were recruited. Participants were recruited from the Greater Boston area and the Brandeis University campus via newspaper, magazine, and facebook advertisements and received monetary compensation. In the current report, we focused on the young adult participants group only ($N = 46$; Mean age = 21.04; $SD = 3.89$).

All participants filled out a brief medical and psychological screening by telephone before testing and were invited to participate only if they met a specific selection criteria: (a) body mass index (BMI) within the reference range between 18 and 30 kg/m$^2$; (b) luteal phase of menstrual cycle at time of participation, for females; (c) absence of psychiatric, endocrine, or cardiovascular diseases, or other specific chronic diseases; (d) no intake of psychoactive drugs, beta-blockers, gonadal steroids (hormonal contraceptives), GCs; (e) non-smoker, and (f) no previous experience with the stress protocol.

Participants were 57% male and 43% female. Forty-eight percent of participants identified as White or Caucasian American, 31% as Asian or Asian American, 4% as Black or
African American, 12% as Other and 5% were Hispanic. BMI ranged from 19.5 to 29.86 kg/m² ($M = 24.7$, $SD = 2.9$) (see Table 1).

**Measures**

**Mindfulness.** At the beginning of the first day of the study day, participants completed the 26-item Self-Compassion Scale (SCS; Neff, 2003). The SCS includes a mindfulness subscale that includes four positively-scored items (e.g., “When I’m feeling down I try to approach my feelings with curiosity and openness”), and four reverse-scored items (e.g., “When I’m feeling down I tend to obsess and fixate on everything that’s wrong.”). Responses to the mindfulness subscale of the SCS were given on a 5-point scale ($1 = $Almost never; $5 = $Almost always). The SCS has demonstrated concurrent, convergent, and discriminant validity, as well as test-retest reliability (Neff et al., 2003, 2007). In the present study, the mindfulness subscale was internally consistent ($\alpha = .80$). On average, participants reported moderate levels of mindfulness ($M = 2.71$; $SD = .46$).

**Body Mass Index.** Height and weight measurements were taken using a tape measurer and a Seca column scale. BMI was computed using the following formula: weight in kg/(height in m $\times$ height in m).

**Blood Collection and Measurements of Interleukin-6 (IL-6) in Plasma**

At baseline (pre-stress) and 30 and 120 min post-stress, whole blood was drawn using a peripheral venous catheter (BD Nexiva IV catherer, Becton-Dickinson, Franklin Lakes, NJ) containing EDTA as an anticoagulant. Initial placement of the catheter was followed by a resting period of 30 min to ensure recovery from potential stress response to catheter placement or
traveling to the laboratory. Blood samples were centrifuged immediately and plasma was then aliquoted and stored at -80 °C.

IL-6 concentrations were determined using a commercial high-sensitivity ELISA (R&D Systems, Oxford, UK). The limit of detection for IL-6 was 0.09 pg/ml with inter- and intra-assay CVs of 9.2% and 5.3%, respectively. Specific procedures are described in detail in Rohleder et al. (2009). IL-6 responses on both dates were computed as delta scores (i.e., IL-6 levels at 120 min post-TSST minutes IL-6 levels at pre-stress baseline). The 120 min time point was chosen because prior research suggests that inflammation peaks approximately 2 h following stress (von Kanel et al., 2006). Peak levels in the present sample were consistent with this pattern.

Because IL-6 response scores on both days showed significant violations of assumption of normality, log-10 transformations were conducted to correct negative skews. Analyses reported below using the IL-6 response variable were performed using the log-10 transformed variables. Parallel analyses performed on the untransformed variables did not differ in direction or significance levels from those reported below.

**Procedure**

Eligible participants were scheduled for laboratory sessions on two consecutive days. All laboratory sessions were scheduled in the afternoon (1:30-6:30pm) to control for circadian variation of stress hormones. Participants were instructed to refrain from eating or drinking anything but water for 1 hour before the laboratory sessions. Written informed consent was obtained prior to participation and ethical approval was granted by the Brandeis University Institutional Review Board.
Each laboratory session lasted approximately three hours and included a resting period followed by exposure to the Trier Social Stress Test (TSST), and blood draws at baseline, 30, and 120 min following the TSST. At the beginning of day 1, mindfulness were assessed using self-report paper-and-pencil questionnaires. Details about these procedures are described below. Other measures not pertinent to the present investigation were also assessed.

**Stress Induction Paradigm**

Acute psychosocial stress was induced using the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), a widely used standardized laboratory stress paradigm. The TSST used in the present consisted of a three minute preparation period, a five minute public speech, and a five minute mental arithmetic task in front of an audience of two judges wearing lab coats and maintaining a neutral evaluative facial expression. The public speech involved describing how one’s personality makes one qualified for a dream job and the mental arithmetic task involved counting backwards from 2043 by increments of 17 on the first study day and from 2011 by 13 on the second study day. Participants were informed that the judges were trained in analyzing verbal and non-verbal behavior and that their performance would be videotapes. The TSST has demonstrated reliability and validity and has been shown to produce strong biological response to stress (Dickerson and Kemeny, 2004).

Immediately following each TSST, participated rated the extent to which they felt “distressed” by the task on a 5-point scale (1= Very slightly or not at all; 5 = Extremely). On average, participants reported feeling moderate psychological distress (TSST1 $M = 2.98$, $SD = 1.31$; TSST2 $M = 2.52$, $SD = 1.17$) (See Table 1).
Results

Preliminary Analyses

Mindfulness was not significantly correlated with age, BMI, or distress at either TSST (all ps > .12) There are no significant gender differences by mindfulness $F(1, 43) = .50$ ($p = .48$). Means and standard deviations for mindfulness, subjective distress, age, and BMI are presented in Table 1.

IL-6 response

We first examined whether exposure to the TSST increased the amount of circulating IL-6. Repeated measures ANOVA indicated that, on average, IL-6 levels increased significantly from baseline to 120 min following both TSSTs: TSST1 $F(1, 45) = 82.71, p < .001$; TSST2 $F(1, 41) = 84.80, p < .001$. IL-6 concentration differed significantly across TSSTs, $F(1, 41) = 5.32, p = .026$ indicating that there was sensitization upon repeated stress exposure. Mean IL-6 levels across the 3 time points on both days are shown in Figure 1 and Table 2.

Mindfulness and TSST1 baseline IL-6

We next performed a linear regression analysis to examine whether mindfulness predicted baseline IL-6 levels on day 1. Mindfulness was not a significant predictor of baseline IL-6, $\beta = .074, t(1, 43) = .50, p = .63$.

Mindfulness and TSST1 IL-6 response
We next performed a linear regression analysis to examine whether mindfulness predicted IL-6 response to TSST 1. Consistent with our hypothesis, mindfulness was a significant negative predictor of day 1 IL-6 response, $\beta = -0.33$, $t(1, 43) = -2.32$, $p = .025$. (see Figures 2 and 3).

Mindfulness remained a significant predictor of TSST1 IL-6 response when controlling for subjective distress, age, gender, and BMI, $\beta = -0.34$, $t(5, 38) = -2.22$, $p = .033$.

**Mindfulness as a predictor of baseline IL-6 for TSST 2**

We performed a regression analysis to examine whether mindfulness predicted baseline IL-6 levels before TSST2. Mindfulness was not a significant predictor of baseline IL-6, $\beta = -0.19$, $t(1, 43) = -1.23$, $p = .23$.

**Mindfulness and TSST 2 IL-6 response**

We next performed a linear regression analysis to examine whether mindfulness predicted IL-6 response to TSST2. Mindfulness was not a significant predictor of TSST 2 IL-6 response, $\beta = -0.07$, $t(1, 43) = -0.465$, $p = .65$.

**Discussion**

In the present study, we examined the relationship between trait mindfulness and IL-6 response to a novel and repeated stressor. Consistent with our hypothesis, we found that trait mindfulness was a negative predictor of IL-6 response to a novel stressor, and mindfulness remained a significant predictor of IL-6 response when controlling for subjective distress, age,
gender, and BMI. Contrary to hypothesis, however, we found that trait mindfulness was not a significant predictor of IL-6 response to a repeated stressor.

These results are consistent with prior research demonstrating the beneficial effects of being mindful in relation to social stress (e.g., Tomfohr, Pung, Mills & Edwards, 2015). For example, consistent with the mindfulness stress-buffering hypothesis, one experiment (Tomfohr et al., 2015) found that individuals who were more mindful showed a lower cortisol response to the Trier Social Stress Task relative to those who were less mindful. In the present study, similar results were found for IL-6 responses to an initial stressor.

Mindfulness was not significantly related to baseline IL-6 levels for either the novel or repeated stressor, suggesting that mindful individuals, at least when they are relatively young in age, are not necessarily lower in chronic low-grade inflammation. However, it is possible that over time less mindful individuals who show strong IL-6 responses to stress will develop low-grade inflammation.

The lack of a significant relationship between mindfulness and IL-6 response to the repeated stressor in this study indicate that mindfulness may be most helpful in the context of novel stressors, which may be perceived as more threatening than familiar stressors due to their unpredictability. However, future research is needed to examine whether there are circumstances under which mindfulness may in fact be beneficial for repeated stress as well.

This study had several limitations worth noting. First, the scale used to measure trait mindfulness was a sub-scale of the Self-Compassion Scale (Neff, 2003) and assesses
mindfulness with only 8 items. Though the scale has been shown to be reliable and valid, future research should use other validated mindfulness scales, such as the Five Factor Mindfulness Questionnaire (Baer, Smith, Hopkins, Krietemeyer, Toney, 2006), to see if the results are the same. Second, mindfulness was not experimentally induced, limiting the causal conclusions that can be drawn. It is possible that individuals who are higher in mindfulness also have less reactive IL-6 responses for reasons other than their mindfulness levels. Third, this study examined a primarily college-age population; future research could examine whether dispositional mindfulness is also beneficial for older participants and those with stress-related illnesses.

In conclusion, our findings support the hypothesis that mindfulness may buffer individuals against inflammatory responses to stress, at least for relatively novel stressors. Interventions aimed at increasing dispositional mindfulness may be especially useful for preventing stress-induced inflammation.
APPENDIX A: TABLES
Table 1.

Descriptive Statistics of Mindfulness, BMI, TSST1 and TSST2 Distress

<table>
<thead>
<tr>
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<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
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<td>Body Mass Index</td>
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<td>TSST2 Distress</td>
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<td>Valid N (listwise)</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>
Table 2.

*Descriptive Statistics of TSST1 and TSST2 IL-6 levels*

<table>
<thead>
<tr>
<th></th>
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<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
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<tr>
<td>Valid N (listwise)</td>
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</table>
Fig. 1. Mean IL-6 levels at baseline and 30 and 120 min post-TSST on days 1 and 2.
Fig. 2. Mindfulness predicting TSST1 IL-6 response.
Fig. 3. Mean TSST 1 IL-6 levels at baseline and 30 and 120 min post-TSST for participants high and low mindfulness (median split).
REFERENCES


