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## EFFECTS OF A SHORT-TERM MINDFULNESS INTERVENTION ON DEPRESSION AND IMMUNE FUNCTION

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David T.R. Berry, Director of Graduate Studies

EFFECTS OF A SHORT-TERM MINDFULNESS INTERVENTION ON  
DEPRESSION AND IMMUNE FUNCTION

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DISSERTATION

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A dissertation submitted in partial fulfillment of the  
requirements for the degree of Doctor of Philosophy in the  
College of Arts and Sciences  
at the University of Kentucky

By  
Erin C. Walsh

Lexington, Kentucky

Director: Dr. Ruth A. Baer, Professor of Psychology

Lexington, Kentucky

2011

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## ABSTRACT OF DISSERTATION

### EFFECTS OF A SHORT-TERM MINDFULNESS INTERVENTION ON DEPRESSION AND IMMUNE FUNCTION

Pro-inflammatory cytokines have been implicated in the pathophysiology and maintenance of depression. This study investigated the effects of a short mindfulness intervention on pro-inflammatory correlates of depression (IL-6 and TNF- $\alpha$ ) and self-reported psychological health. Sixty-four college females were assigned to a four-week mindfulness training group or a contact-control group. Cytokines and psychological health were assessed at baseline, post-treatment, and 3-month follow-up (mindfulness group only). IL-6 and TNF- $\alpha$  significantly decreased from baseline to post-treatment in the mindfulness group only; these changes were sustained at 3-month follow-up. No between-group differences in psychological health emerged. Although reductions in pro-inflammatory cytokines in the mindfulness condition were not attributable to psychological changes, they may serve to protect against the development of future depressive episodes.

Keywords: Mindfulness, yoga, pro-inflammatory cytokines, depression, immunity

Erin Celine Walsh  
Student's Signature

December 21, 2011  
Date

EFFECTS OF A SHORT-TERM MINDFULNESS INTERVENTION ON  
DEPRESSION AND IMMUNE FUNCTION

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## TABLE OF CONTENTS

Acknowledgements.....	iii
List of Tables.....	vi
List of Figures.....	vii
Section One: Introduction.....	1
Associations between Depression and Innate Immunity.....	1
Effects of Mindfulness-Based Practices on Psychological States and Functioning.....	2
Effects of Mindfulness-Based Practices on Immunity.....	3
Summary and Significance.....	5
Study Aims and Hypotheses.....	5
Aim 1.....	6
Hypothesis 1.....	6
Hypothesis 2.....	6
Hypothesis 3.....	6
Aim 2.....	6
Hypothesis 4.....	6
Section Two: Method.....	7
Overview of Study Design.....	7
Participant Recruitment and Allocation.....	7
Initial Screening.....	7
Phone Screening.....	7
Group Assignment.....	8
Study Procedures.....	8
Baseline Assessment (Session 1) .....	8
Mindfulness Training Sessions.....	9
Body Scan (Session 1) .....	9
Sitting Meditation (Sessions 3 & 5) .....	9
Yoga (Session 4) .....	9
Contact-control Sessions.....	9
Post-treatment Assessment.....	10
3-month Follow-up Assessment.....	10
Procedures for Enhancing Recruitment and Retention.....	10
Psychological Measures.....	10
Depressive Symptoms.....	11
Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I) .....	11
Psychological Symptoms.....	11
Emotion Regulation.....	12
Physical Symptoms.....	12



Mindfulness.....	12
Well-being.....	12
Biological Measures.....	13
Salivary Cytokine Samples.....	13
Potential Covariates.....	13
General Health Questionnaire.....	13
Data Screening.....	14
Statistical Analyses.....	14
Section Three: Results.....	17
Participant Flow.....	17
Baseline Data.....	17
Aim 1: Treatment Effects.....	17
Condition Effects on Salivary Cytokines.....	17
Condition Effects on Self-Report Outcomes.....	18
Longitudinal Outcomes within the Mindfulness Training Condition.....	19
Aim 2: Potential Mediators of Cytokine Outcomes.....	20
Section Four: Discussion.....	29
Limitations.....	32
Conclusions.....	33
References.....	35
Vita.....	42

## LIST OF TABLES

Table 3.1, Descriptives by Sample.....	21
Table 3.2, Means and Standard Deviations for Outcome Variables at Baseline and Post-treatment by Condition.....	22
Table 3.3, Estimates for Poisson Regression Models Predicting IL-6 and TNF- $\alpha$ Using PROC MIAnalyze.....	24

## LIST OF FIGURES

Figure 2.1, Flowchart for participant progress (based on the consolidated standards of reporting trials [CONSORT] recommendations).....	16
Figure 3.1, Effect of Condition on Change in IL-6 Pre-to-Post-Treatment.....	25
Figure 3.2, Effect of Condition on Change in TNF- $\alpha$ Pre-to-Post-Treatment.....	26
Figure 3.3, Effect of Condition on Change in Observe Pre-to-Post-Treatment.....	27
Figure 3.4, Effect of Condition on Change in Nonjudging Pre-to-Post-Treatment.....	28

## Section One: Introduction

Depression affects approximately 20.9 million US adults (9.5%) each year (NIMH, 2008). Individuals with depression are impaired in many domains of functioning and are at higher risk for disease morbidity (Cuijpers & Smit, 2002; Rudisch & Nemeroff, 2003). Although depressive episodes can be effectively treated and may resolve without treatment, the high risk of relapse associated with unipolar major depression necessitates the identification of effective prevention and relapse-prevention strategies (Gotlib & Hammen, 2002). Adults who have experienced one major depressive episode have a 60% likelihood of recurrence, and this risk increases with each episode, such that those who have a history of three episodes have a 90% likelihood of experiencing a fourth (DSM-IV-TR, 2000).

### *Associations between Depression and Innate Immunity*

Nonspecific immune, behavioral, and physiological changes occur with the presence of infection or inflammation (Dantzer, 2001; 2006). Such illness responses include fever, fatigue, difficulties with concentration, lack of interest in surroundings, reduced food intake, and behavioral disengagement (Dantzer, 2001; 2006; Kemeny, 2006). Collectively, these symptoms are referred to as “sickness behavior”. Sickness behavior is considered a normal and adaptive response to acute immune threats (Dantzer 2001; 2006; Dantzer et al., 2006). It enhances survival by allowing the organism to conserve energy and disengage from situations or environments that are potentially harmful, thus promoting numerous healing and restorative processes (Kemeny, 2006). Although sickness behavior is regarded as an adaptive mechanism in particular contexts, it is also believed to contribute to the pathophysiology and maintenance of depression (Dantzer, 2001, 2006).

Sickness behavior is triggered by pro-inflammatory cytokines, including interleukins (e.g. IL-1, IL-6), interferons, and Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) (Dantzer, 2001, 2006; Dantzer et al., 2006). Pro-inflammatory cytokines are often produced by innate immune cells (Dantzer, 2001; Dinan, 2008) and have been related to depression in the following ways: 1) Increased levels of circulating pro-inflammatory cytokines are observed in individuals with depressive symptomatology (Howren et al., 2009; Zorrilla et al., 2001); 2) Individuals with an underlying inflammatory disease

experience symptoms of depression, likely due to disease-related release of pro-inflammatory cytokines (Kemeny, 2006; Capuron et al., 2006); 3) Patients undergoing immunotherapy to treat cancer report psychological and behavioral changes consistent with depression upon short-term administration of a pro-inflammatory cytokine (Capuron et al., 2002) - especially individuals identified as “at-risk” (Capuron & Ravaud, 1999), 4) Animals injected with pro-inflammatory cytokines exhibit behavioral signs of sickness (Dantzer et al., 2006), and 5) Administration of anti-depressants to animals reduces levels of circulating pro-inflammatory cytokines and sickness behavior (Pollak & Yirmiya, 2002). Although this evidence is suggestive that pro-inflammatory cytokines *cause* depression, the directionality of this relationship is much more complex. The positive association between pro-inflammatory cytokines and depression is likely the result of a bidirectional process, in which immune products alter depressive symptoms, and vice versa (Howren et al., 2009). Finally, and most pertinent to human research on depression, a recent meta-analysis shows that higher concentrations of IL-6 and TNF- $\alpha$  are more likely to be found in depressed participants than other pro-inflammatory markers (Dowlati et al., 2010).

### ***Effects of Mindfulness-Based Practices on Psychological States and Functioning***

Mindfulness is a form of self-regulation of attention that originates from Eastern spiritual traditions and is conceptualized as nonjudgmentally bringing attention to internal and external events that arise within the present moment (Kabat-Zinn, 1994). Sustained practice of mindfulness is thought to enhance positive personal qualities, such as awareness, insight, wisdom, and compassion, and to reduce suffering (Goldstein, 2002; Kabat-Zinn, 2003). Although historically rooted in Buddhism, mindfulness has been adopted in secular form as an intervention for numerous psychological and physical ailments in Western settings (Baer, 2003).

One of the most cited programs in mindfulness training is mindfulness-based stress reduction (MBSR), developed by Kabat-Zinn (1982, 1990). MBSR is typically conducted in a group setting over the course of 8 weeks, with up to 30 members per group and weekly sessions lasting 2.0 - 2.5 hours. Versions with fewer and shorter sessions have been reported, with no loss of efficacy (Carmody & Baer, 2009). In standard MBSR, patients are encouraged to practice mindfulness meditation exercises at

home for 45 minutes per day, six days a week during the intervention. Several studies have reported shorter practice times (20 minutes per day), again with no loss of efficacy (Carmody & Baer, 2009). Patients in MBSR are taught to observe their experiences, including uncomfortable or distressing thoughts and sensations, in a nonjudgmental, non-avoidant fashion. Practicing mindfulness is thought to increase patients' tolerance of unpleasant physical, cognitive, and emotional states through processes of repeated exposure or decentering (Baer, 2003; Keng, Smoski, & Robins, 2011). Mindfulness practices taught in this program include hatha yoga, the body scan, sitting and walking meditations, and activities for cultivating mindfulness in daily life (Baer, 2006).

Studies have shown mindfulness-based interventions to be efficacious for a variety of disorders including depression (Baer, 2003; Kabat-Zinn, 2003). Self-reported benefits include reductions in mood disturbance, anxiety, difficulties with emotion regulation, and general psychological symptoms, and increases in psychological well-being (see Keng et al., 2011 for a review). Furthermore, mindfulness-based cognitive therapy (MBCT), a program closely based on MBSR that integrates mindfulness techniques with cognitive therapy, has demonstrated efficacy in the prevention of depression relapse for a period of one year following treatment, with individuals who have experienced three or more previous episodes (Teasdale et al., 2000; Segal, 2002; Ma & Teasdale, 2004). Several studies have also shown that administering MBCT to acutely depressed participants yields clinically significant improvements (Eisendrath et al., 2008; Finucane & Mercer, 2006; Kenny & Williams, 2006; Kingston et al., 2007; Ree & Craige, 2007).

### ***Effects of Mindfulness-Based Practices on Immunity***

Investigations examining the effects of mindfulness practice on immune and neuroendocrine function have yielded promising results. Davidson et al. (2003) reported that participation in an 8-week MBSR program enhanced antibody titers to influenza vaccination in a sample of healthy but stressed adults. Creswell et al. (2009) showed that patients diagnosed with HIV did not exhibit declines in CD4+ T lymphocytes upon completion of an 8-week MBSR intervention, whereas those in a control condition showed the expected declines. Carlson et al. (2003) and Witek-Janusek et al. (2008) found significant reductions in pro-inflammatory cytokines among cancer patients

enrolled in an 8-week MBSR course, and these results were sustained at a one-year follow-up (Carlson et al., 2007). Pace et al. (2009) and Tang et al. (2007) demonstrated that short-term meditation interventions were related to changes in immune and neuroendocrine functioning in healthy student samples following an experimental stressor. Specifically, participants who received mindfulness training showed reductions in IL-6 (Pace et al., 2009) and cortisol (Tang et al., 2007), and increases in salivary IgA (Tang et al., 2007). Short-term interventions consisted of two 50-minute sessions per week over the course of 6-weeks (Pace et al., 2009), and one 20-minute session for five consecutive days (Tang et al., 2007). Lastly, some studies report that treatment adherence or home practice is positively associated with immune outcomes (Creswell et al., 2009; Pace et al., 2009), while others have not observed this effect (Carlson et al., 2007).

Although results are encouraging, these investigations are not without methodological limitations. Some studies have failed to include control conditions (Carlson et al., 2003; 2007), which significantly limits conclusions regarding the effects of MBSR on immunity. Many studies have neglected to assess participants longitudinally (Davidson et al., 2003; Tang et al., 2007, Witek-Janusek et al., 2008, Creswell et al., 2009, Pace et al., 2009), making it difficult to evaluate if MBSR training has an enduring impact on immune processes. Pertinent to the current study, Pace et al. (2009) reported significantly reduced levels of IL-6 in participants in a short-term mindfulness intervention following an experimental stressor. However, this effect was only seen in participants who reported high levels of meditation practice outside of sessions. Furthermore, although this group showed a significant change from pre-treatment, no significant difference in level of IL-6 was observed between the meditation group and an attention-control group following treatment. The lack of between-group effect may have been the result of using a non-psychologically vulnerable population or teaching a meditation practice not common to MBSR structure (i.e., compassion meditation). Finally, while investigations examining the effects of mindfulness training on immunocompromised populations (e.g. HIV, cancer) are necessary and informative, they shed little light on the effects of mindfulness on immunity in otherwise healthy people with depression. Although diseases such as cancer share similarities with

depression, in that both conditions elevate circulating pro-inflammatory cytokines, it is difficult to interpret immunological findings from these populations. That is, reductions in cytokines may be attributed to cancer recovery, reductions in depression, or both. For this reason, it is necessary to investigate immunological changes following mindfulness training in a depressed but immunocompetent (or otherwise healthy) sample.

### ***Summary and Significance***

Investigations examining the effects of mindfulness practice on previously and acutely depressed individuals show clinically significant improvements in functioning across a number of domains. In addition, mindfulness practice appears to contribute to optimal immune functioning in both normal and clinical populations. However, it remains unknown how mindfulness practice relates to immune changes in an immunocompetent depressed population. There is also limited research on the long-term benefits participants receive following mindfulness-based interventions.

The current study investigated the effects of a short-term mindfulness intervention on psychological and immune functioning in a sample with moderate depressive symptoms. This study assessed all participants from baseline to post-treatment. Participants in the mindfulness intervention were further evaluated at a 3-month follow-up point to assess maintenance of treatment gains. Recent evidence (Tang et al., 2007; Pace et al. 2009) has demonstrated the potential benefits of short-term meditation practice on attention, emotion, and immunity, and showed that number of in-class hours is not strongly related to psychological outcomes in clinical and non-clinical samples (Carmody & Baer, 2009). These findings suggest that short-term mindfulness interventions may provide benefits equivalent to those received in standard 8-week courses.

This study was designed to improve upon previous investigations of immune function and mindfulness training by: 1) monitoring an immune variable relevant to depressive symptoms, 2) longitudinally assessing immune and psychological outcomes, 3) recruiting a psychologically vulnerable yet immunocompetent population, and 4) evaluating a mechanism of action responsible for reducing levels of pro-inflammatory immune correlates of depression.

### ***Study Aims and Hypotheses***



**Aim 1.** To assess the longitudinal effects of a short-term mindfulness intervention on immunity and psychological health in a sample with moderate depressive symptoms.

*Hypothesis 1:* Compared to a contact-control group, individuals participating in the mindfulness intervention will show reductions in two pro-inflammatory immune correlates of depression: interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ), at immediate post-intervention assessment.

*Hypothesis 2:* Compared to a contact-control group, individuals participating in the mindfulness intervention will experience reductions in self-reported depression, psychological and physical symptoms, and emotional dysregulation, and report improvements in psychological well-being and the tendency to be mindful in daily life at immediate post-intervention assessment.

*Hypothesis 3:* For participants in the mindfulness intervention, improvements in immunity and psychological health are expected to remain relatively stable at a 3-month follow-up assessment.

**Aim 2.** To examine a possible mechanism of action responsible for reduction in pro-inflammatory immune correlates of depression.

*Hypothesis 4:* At immediate post-intervention assessment, self-reported depression is expected to mediate the relation between treatment condition (mindfulness training vs. contact-control) and pro-inflammatory immune correlates of depression (IL-6 and TNF- $\alpha$ ).

Although recent commentary (Howren et al., 2009) suggests that the association between depression and pro-inflammatory cytokines is likely the result of a bidirectional process, this study proposes that reductions in self-reported depression will lead to reductions in IL-6 and TNF- $\alpha$ . Because mindfulness training targets disruptive cognitive and affective states through exercises emphasizing non-judgmental observation and acceptance, it seems reasonable to conclude that depressive symptoms will initially decrease, thereby diminishing potential mediators of pro-inflammatory immune activation (e.g., stress hormones).

## Section Two: Method

### **Overview of Study Design**

This was a longitudinal intervention study of young women with moderate depressive symptoms. Participants were assigned to either a mindfulness training or a contact-control condition. Over the course of the investigation, participants completed three separate assessments: (a) before treatment initiation (baseline), (b) following treatment completion (6-week post-treatment assessment), and (c) 3-months after treatment completion (3-month follow-up; mindfulness training only). At each time point, self-report data and salivary cytokines (IL-6, TNF- $\alpha$ ) were assessed. Data were collected between September 2010 and June 2011. Complete information on participant flow is presented in the Results section (based on the consolidated standards of reporting trials [CONSORT] recommendations) and can be viewed in Figure 2.1.

### **Participant Recruitment and Allocation**

***Initial Screening.*** At the beginning of the Fall 2010 and Spring 2011 semesters, all students in introductory psychology at the University of Kentucky were offered the opportunity to participate in an in-class screening session for course credit. During this session students filled out an approved screening questionnaire, the Center for Epidemiological Depression Scale (CES-D; Radloff, 1977), assessing their current level of depression. Students who endorsed moderate symptoms of depression (scores ranging from 16-24) were contacted about their interest in participating in the current study and invited to complete a phone screening with the PI.

***Phone Screening.*** During the phone screening, potential participants were informed that the purpose of the study was to examine the effects of mindfulness meditation and yoga on health and well-being. Potential participants were told that they would have to complete the phone screening to determine if they met eligibility criteria for the study. Eligibility criteria included: (a) endorsement of moderate depressive symptoms based upon self-report screening measure, (b) ages 18-25 years old, (c) female, (d) absence of suicidal ideation and suicide intent/plan, (d) absence of specific psychiatric disturbance (i.e., psychosis, bipolar disorder, PTSD), (e) absence of alcohol/drug dependence, (f) absence of immune disease or disorder (e.g., lupus, HIV), (g) absence of pregnancy, (h) not currently taking psychotropic agents (i.e., anti-depressants,

anxiolytics, anti-psychotics), statins, beta-blockers, anti-hypertensives, and nonsteroidal anti-inflammatory drugs, (i) willingness to be randomly assigned to the mindfulness or contact-control condition, and (j) ability to read, write and understand English.

**Group Assignment.** Students who met criteria for the study following the phone screening were assigned to a group. Four groups were run each semester (Fall 2010, Spring 2011) to maximize the number of students who could be included in the study. Groups typically took place in the early mornings (i.e., 8am or 9am) to increase the likelihood that participants would adhere to study procedures on assessment days (see below for more detail). Due to students' schedule constraints, unrestricted randomization was not possible. Therefore, group allocation procedures were as follows: a) If a student could participate in any of the available group times, she was randomized to a group; b) If a student could only participate in a restricted number of groups, she was randomized to a group that fit within her schedule; and c) If a student could only participate in one group due to schedule constraints, she was assigned to that particular group. The PI and students were blind to condition until all eligible participants were enrolled each semester. Once study enrollment was complete, the PI randomized condition for each group (i.e., mindfulness training or contact-control) such that there were two mindfulness training and two contact-control groups offered each semester. Participants learned of their condition status at the baseline assessment. All randomization procedures were completed with the assistance of an online random number generator ([www.random.org](http://www.random.org)).

## **Study Procedures**

**Baseline Assessment (Session 1).** At the agreed upon day and time, the PI met with each group, consisting of no more than 20 members each. After completion of informed consent procedures, participants were asked to complete a packet of self-report measures assessing psychological and physical health status. Additionally, participants were asked to provide a saliva sample for assessment of immune function. Following collection of these measures, the PI provided participants with a brief overview of the study, including information on what they would be asked to do each week. Contact-control participants were informed that they could participate in a short-term mindfulness course the following semester, if interested. Completion of the baseline assessment took 50 minutes.

***Mindfulness Training Sessions.*** All mindfulness training sessions were completed in weeks 2-5 following baseline assessment. Participants met in groups with the PI once per week to practice a specific mindfulness exercise. Each session included 35 minutes of mindfulness practice and 15 minutes of discussion (e.g., discussion of weekly experience, applicability of practice to daily life). At the end of each session, participants were given a take-home CD of the mindfulness practice reviewed that week. Although it was optional, participants were encouraged to practice the mindfulness exercise while at home. They were provided with daily tracking logs to record the number of minutes they attempted any formal meditation practice. Only two participants attempted at-home practice over the course of the study. Due to insufficient participation, this variable was not included in any final analyses.

- **Body Scan (Session 2):** The PI led participants through an exercise asking them to non-judgmentally observe sections of their body (e.g., toes, thighs, back, neck). Emphasis was placed on examining the sensations present in each location. Practice began and ended with attention to the breath, and is described in Segal et al. (2002).
- **Sitting Meditation (Sessions 3 & 5):** The PI led participants through an exercise asking them to non-judgmentally observe: 1) the sensations of breathing, 2) the sensations present in the body, 3) the content of thoughts, 4) the presence of emotions, and 5) the sounds in the environment. Practice began and ended with attention to the breath, and is described in Segal et al. (2002). As sitting meditation receives considerable attention in mindfulness-based interventions, two sessions were devoted to this exercise.
- **Yoga (Session 4):** The PI led participants through light stretching exercises described in Kabat-Zinn (1990). Participants were asked to focus nonjudgmentally on the particular sensations associated with bodily movement. Modified poses were provided to participants who were unable to perform the full range of movement for a specific yoga pose. Practice began and ended with focus on the breath.

***Contact-control Sessions.*** Participants in the contact-control groups were asked to fill out questionnaires unrelated to the proposed study, in weeks 2-5, following baseline assessment. Participants met in groups once per week, and each session lasted 50 minutes.

***Post-treatment Assessment.*** During week 5, participants were reminded of the upcoming post-treatment assessment. Participants were asked to return the following week at the agreed upon date, time, and location to complete assessment measures equivalent to those collected during the baseline session. At this assessment, participants in the contact-control groups were invited to take part in a short-term mindfulness course the following semester. Interested students were told to contact the PI if they wished to participate. No contact-control participants opted to receive mindfulness training once the study was complete. Completion of the post-treatment assessment session took 50 minutes.

***3-month Follow-up Assessment (mindfulness training ONLY).*** One month prior to the 3-month follow-up, the PI sent an email to mindfulness training participants reminding them of the final follow-up assessment. Participants met with the PI in small groups or individually to complete self-report and salivary assessment measures. These sessions were held approximately three months following completion of the 6-week follow-up and lasted 40-50 minutes. A total of 21 participants attended this session and provided complete follow-up data. An additional three participants who were willing to complete the 3-month assessment yet could not attend due to being out of state were mailed a packet of self-report questionnaires but did not complete the salivary assessment. These participants filled out and returned the packets within 2 weeks.

Individuals in the contact-control group were not evaluated at the 3-month follow-up. Based upon the University of Kentucky IRB requirements, participants in the contact-control group were offered the described mindfulness intervention immediately following the 6-week post-treatment assessment. Because we could not predict whether participants would engage in the treatment at the start of the following semester, and thereby confound potential results, we chose not to assess them at this time point.

***Procedures for Enhancing Recruitment and Retention.*** To increase recruitment and retention, participants received course credit for the baseline assessment and each intervention session attended, and \$20 was awarded to participants who attended all of these sessions. In addition, \$20 was offered for completion of the post-treatment and 3-month follow-up sessions.

## **Psychological Measures**

***Depressive Symptoms.*** The *Center for Epidemiologic Depression Scale (CES-D)* is a 20-item inventory of depressive symptoms (Radloff, 1977). The CES-D asks participants to rate their mood, thoughts, and behavior during the previous week on a 4-point Likert scale. CES-D items were scored such that higher total scores were indicative of greater depressive symptoms. For the current study, scores ranging from 16-24 were used to screen for moderate symptoms of depression (Greden & Schwenk, 1997). The CES-D was also administered at all study time points to monitor changes in depression severity. For the present sample, internal consistencies were as follows: Baseline assessment ( $\alpha = .80$ ), post-treatment assessment ( $\alpha = .79$ ), 3-month follow-up ( $\alpha = .65$ ).

***Structured Clinical Interview for DSM-IV-TR Axis-I Disorders (SCID-I).*** The SCID-I is a semi-structured interview used to diagnose the major Axis I DSM-IV-TR disorders (First et al., 1994). The SCID-I can be administered in full (i.e. all modules) or adapted to assess particular diagnostic classes of interest. For the current study, the following modules were used during the phone screening to identify students who were ineligible due to the presence of particular disorders: Major Depressive Disorder (for severe symptom severity and suicidality), Substance Use Disorders (for alcohol/drug dependence), Bipolar I/II Disorder (for symptoms of severe depression or mania), and PTSD (for symptoms of severe anxiety and post-traumatic stress). Additionally, items measuring extreme psychotic symptoms were used to screen out participants with psychosis.

***Psychological Symptoms.*** The Brief Symptom Inventory (BSI) is a 53-item self-report instrument that assesses current psychopathology. It contains nine symptom dimensions (Somatization, Obsessive-Compulsiveness, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Ideation, and Psychoticism) and a Global Severity Index (GSI) that represents overall psychological distress. Only the GSI was used in the present study. The BSI has demonstrated high internal consistency and significant associations with measures of similar constructs. Participants completed the BSI at all three assessments. Internal consistencies for GSI were as follows: Baseline assessment ( $\alpha = .91$ ), Post-treatment assessment ( $\alpha = .89$ ), 3-month follow-up ( $\alpha = .91$ ).

**Emotion Regulation.** The *Difficulties in Emotion Regulation Scale* (DERS) is a 36-item measure designed to assess deficits in emotion regulation (Gratz & Roemer, 2004). It includes six subscales: Lack of Acceptance of Emotional Responses, Lack of Emotional Awareness, Lack of Emotional Clarity, Limited Access to Effective Emotion Regulation Strategies, Lack of Impulse Control, and Difficulties Engaging in Goal-Directed Behavior when experiencing distress. Higher scores indicate greater emotion dysregulation. In the development sample, the DERS demonstrated good test-retest reliability and significant associations with measures of similar constructs. Participants completed the DERS at all three assessments. Internal consistencies for subscales were as follows: Baseline assessment ( $\alpha$  ranging from .55-.88), Post-treatment assessment ( $\alpha$  ranging from .74-.90), 3-month follow-up ( $\alpha$  ranging from .79-.90).

**Physical symptoms.** Self-perceived health was assessed with the *Pennebaker Inventory of Limbic Languidness* (PILL; Pennebaker, 1982). The PILL is a 54-item scale that measures the frequency of experiencing common physical symptoms and sensations (e.g., indigestion, headaches, nausea). The PILL demonstrates high internal consistency and has been shown to correlate moderately with similar symptom scales (Pennebaker, 1982). For the present study, the PILL was completed at all three assessments and internal consistencies were as follows: Baseline assessment ( $\alpha = .91$ ), Post-treatment assessment ( $\alpha = .92$ ), 3-month follow-up ( $\alpha = .96$ ).

**Mindfulness.** The *Five Facet Mindfulness Questionnaire* (FFMQ) is a 39-item self-report questionnaire designed to measure five facets of mindfulness: Observing, Describing, Acting With Awareness, Nonjudging of experience, and Nonreactivity to inner experience (Baer et al., 2006). The FFMQ has shown good internal consistency for the five facets, and has demonstrated convergent and discriminant validity with several measures of related constructs. Furthermore, all facets appear to be distinct from one another, exhibiting only moderate inter-correlations. The FFMQ was completed at all three assessments and internal consistencies for subscales were as follows: Baseline assessment ( $\alpha$  ranging from .81-.86), Post-treatment assessment ( $\alpha$  ranging from .65-.91), 3-month follow-up ( $\alpha$  ranging from .74-.88).

**Well-being.** The *Scales of Psychological of Well-Being* (PWB) assess six subscales: Self-Acceptance, Environmental Mastery, Positive Relations with Others,

Personal Growth, Purpose in Life, and Autonomy (Ryff, 1989). Higher scores reflect greater well-being. Validity of the PWB scores is evidenced by significant positive correlations with measures of positive functioning (i.e. life satisfaction, self-esteem) and negative correlations with measures of negative functioning (i.e. depression) (Ryff, 1989). Participants completed the PWB at all three assessments. Internal consistencies for subscales were as follows: Baseline assessment ( $\alpha$  ranging from .64-.81), Post-treatment assessment ( $\alpha$  ranging from .73-.84), 3-month follow-up ( $\alpha$  ranging from .72-.86).

### **Biological Measures**

*Salivary cytokine samples.* Although typically assessed in serum levels, studies show that IL-6 and TNF- $\alpha$  can be detected in whole unstimulated saliva (Sjogren et al., 2006; Tishler et al., 1999; Pezelj-Ribaric et al., 2004), which is less invasive to collect than serum. For the current study, the “passive drool” method was used to collect saliva samples. Twenty-four hours prior to each saliva sample collection, participants were asked to abstain from caffeine, tobacco, and alcohol products, illicit drugs, and exercise, until the assessment was complete (e.g., caffeine has immunosuppressive effects on pro-inflammatory cytokines, see Horrigan et al., 2006). To collect whole, unstimulated saliva, participants were asked to rinse their mouths with bottled water for 30 seconds and immediately dispose of the water into a paper cup, which was subsequently thrown away. They were then instructed to wait 10 minutes before filling a small tube with 2-5ml of their saliva (Sarstedt AG & CO). Saliva was initially stored for 1-4 weeks at  $-25^{\circ}\text{C}$  before being transferred to  $-80^{\circ}\text{C}$  before assay. Immunoassays were performed at the Clinical Research Development and Operations Center (CR-DOC) at the University of Kentucky in Lexington, KY.

### **Potential Covariates**

*General health questionnaire.* Demographic information (age, race/ethnicity, marital status, current grade, religious affiliation), history of psychotherapy, and history of mindfulness practice were collected at baseline assessment. At all assessment points, participants reported on health-related information, including: height and weight (to calculate BMI); history of health problems (e.g., allergies, cardiac illness, cancer, sleep disturbance); medications; illness (e.g., flu, cold); injury; vaccination status;



hospitalizations or surgeries; oral contraception use; menstrual cycle day/phase; oral health; caffeine, alcohol, and drug use; and amount of physical activity.

The purpose of assessing this information was to control for *potential* variables that might affect levels of circulating pro-inflammatory cytokines. Recent commentary (Segerstrom, 2009; O'Connor et al., 2009) recommends not controlling for variables unless there is a strong theoretical reason to do so. Segerstrom (2009) argues that overfitting a model will have adverse effects on statistical power, results, and replicability. Therefore, the only a priori variable that was entered as a covariate was oral contraception use. For the remaining variables, correlations with IL-6 and TNF- $\alpha$  were computed. Only variables that were significantly correlated with salivary cytokines were included as covariates in subsequent analyses.

### **Data Screening**

Several dependent variables did not meet the regression assumption of normality (i.e., the skewness for a given dependent variable was more than three times the standard error of its skewness). In each such case, an attempt was made to achieve normality using nonlinear transformations (i.e., square root, log 10). For the following variables, square root transformations were adequate for achieving normality: CES-D and Lack of Emotional Clarity (DERS). For Lack of Impulse Control (DERS) and Lack of Acceptance of Emotional Responses (DERS), log 10 transformations were required to achieve normality. The following variables followed a poisson distribution and could not be transformed to normality using a nonlinear transformation: IL-6 and TNF- $\alpha$ . The remaining outcome variables were normally distributed. Between-person covariates (e.g., BMI) were always grand-mean centered for use as predictors.

### **Statistical Analyses**

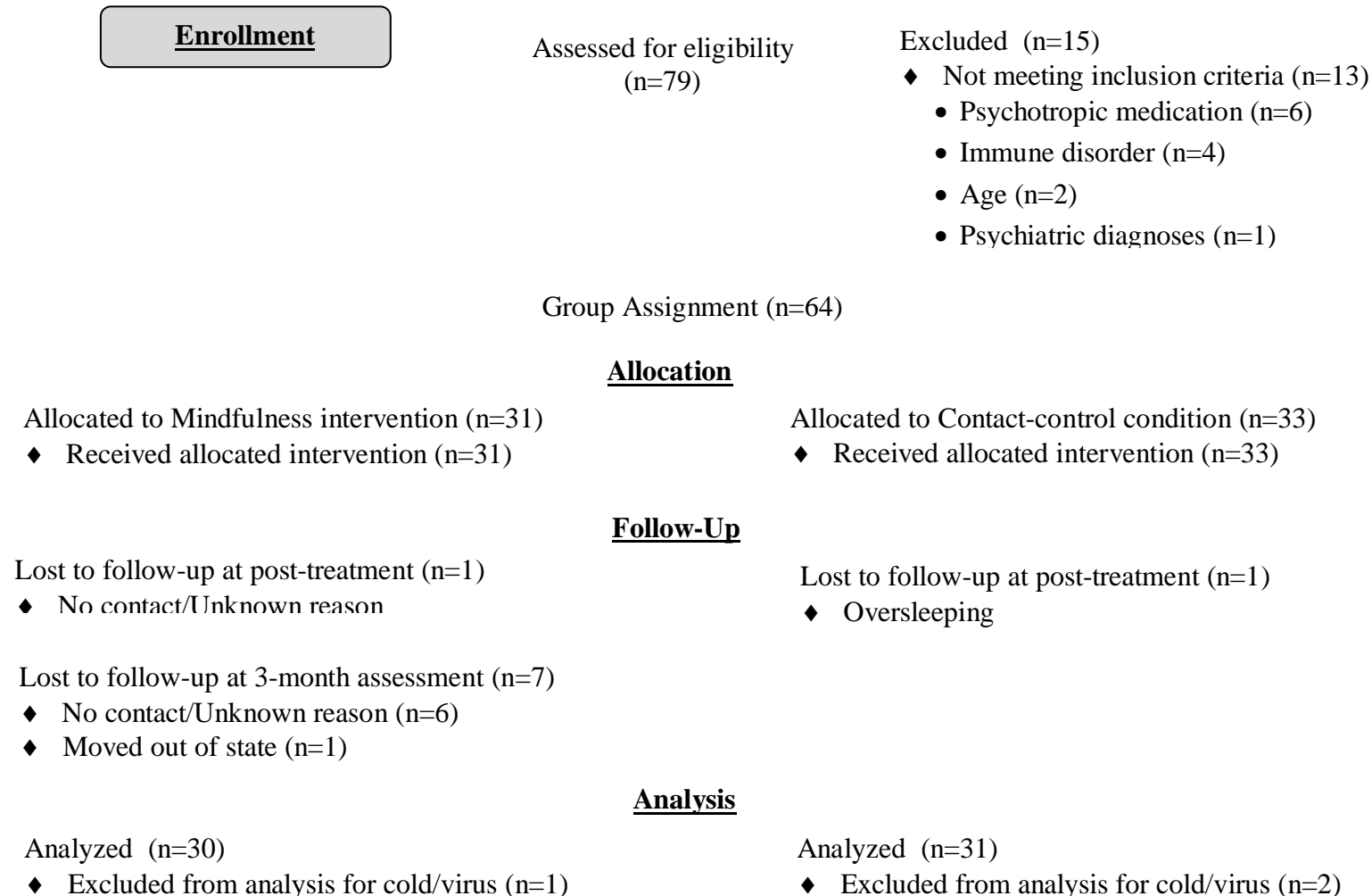
Because some outcome data were missing at random, missing values were imputed using multiple random imputation in SAS PROC MI. To test the prediction that mindfulness training would be associated with lower levels of symptoms at post-treatment assessment controlling for baseline levels, regression models were fit in SAS PROC REG with baseline levels of the relevant outcome measure in step 1, and condition (with mindfulness condition coded as 1, and contact-control condition coded as 0) in step 2. For outcome variables that appeared to follow a poisson distribution and could not be

transformed to normality using a nonlinear transformation, similar regression models were fit in SAS PROC GENMOD specifying a poisson distribution for the dependent variable. Where significant effects of condition were not found, paired sample t-tests were also carried out to determine whether the outcome variable changed pre-post intervention in the whole sample, regardless of condition. Analyses were carried out on each of 20 data sets, imputed using SAS PROC MI, and results were combined using SAS PROC MIANALYZE, which provides 95% confidence intervals for model estimates.

To test the prediction that improvements in pro-inflammatory cytokine levels and psychological health would remain significant at the 3-month follow-up for those in the mindfulness intervention, two paired sample t-tests were carried out for each outcome that showed 1) significant changes from baseline to post-intervention in the full sample, or 2) a significant effect of the mindfulness condition on changes from baseline to post-intervention. The first paired sample t-test addressed whether the difference between baseline and post-intervention assessments was significant within the mindfulness condition, and the second addressed whether the difference between baseline and 3-month follow-up assessments was significant in the mindfulness condition.

Figure 2.1

Flowchart for participant progress (based on the consolidated standards of reporting trials [CONSORT] recommendations)



## Section Three: Results

### **Participant Flow**

Over the course of the study, 79 female students were assessed for study eligibility. Figure 2.1 provides information on accrual and retention of study participants (based on the consolidated standards of reporting trials [CONSORT] recommendations). Fifteen students from the initial pool of 79 were excluded for not meeting inclusion criteria (n=13) or were not interested in participating in the study once contacted (n=2). Sixty-four students met eligibility criteria and were assigned to either a mindfulness training group (n=31) or a contact-control group (n=33). At the post-treatment assessment, 62 participants had completed the study (mindfulness training=30; contact-control=32). One participant dropped out for unknown reasons while the other missed the assessment due to oversleeping. Twenty-four participants in the mindfulness group completed the 3-month follow-up assessment (retention=77%). Out of the seven participants who withdrew from the study, six did not respond to the PI's queries to return for the final follow-up session, and one reported she was not able to participate due to moving out-of-state. Participants who completed the 3-month follow-up were similar to those who did not complete the 3-month follow-up with respect to age, race/ethnicity, current grade, marital status, and religious affiliation (all  $p$ 's > .10).

### **Baseline Data**

Descriptive statistics for the sample by condition can be found in Table 3.1. There were no significant differences in age, race/ethnicity, current grade, or religious affiliation between the two conditions (age:  $t(62) = -.18, p = .86$ ; race:  $X^2(2) = 2.19, p = .33$ , current grade:  $X^2(2) = 1.44, p = .49$ , religious affiliation:  $X^2(2) = 1.24, p = .74$ ). No significant correlations were found between these demographic variables and baseline levels of IL-6 or TNF- $\alpha$ . Descriptives for outcome variables at baseline and post-treatment for the full sample and within each condition can be found in Table 3.2.

### **Aim 1: Treatment Effects**

#### *Condition Effects on Salivary Cytokines*

Estimates and 95% confidence intervals for poisson regression models predicting the effect of condition on cytokine levels at post-treatment controlling for cytokine levels at baseline are presented in Table 3.3. As noted earlier, oral contraceptive use was

controlled for a priori. Upon inspection of correlations between levels of IL-6, TNF- $\alpha$ , and variables measured in the general health questionnaire, only one significant correlation emerged: TNF- $\alpha$  and BMI were positively correlated. Therefore, oral contraceptive use was controlled for in the model predicting IL-6, and both oral contraceptive use and BMI (standardized) were controlled for in the model predicting TNF- $\alpha$ . Results indicated that, controlling for baseline levels of IL-6, participation in mindfulness training predicted lower IL-6 at the post-treatment assessment ( $B_{\text{CONDITION}} = -.61, SE = .17, 95\% \text{ CI: } -.95 \text{ to } -.26, p = .0006$ ). See Figure 3.1 for a graph of condition effects on IL-6 from baseline to post-treatment. Likewise, controlling for baseline levels of TNF- $\alpha$ , participation in mindfulness training was associated with lower levels of TNF- $\alpha$  at post-treatment ( $B_{\text{CONDITION}} = -.37, SE = .23, 95\% \text{ CI: } -.86 \text{ to } -.12, p = .04$ ). See Figure 3.2 for a graph of condition effects on TNF- $\alpha$  from baseline to post-treatment.

#### *Condition Effects on Self-Report Outcomes*

Estimates and 95% CIs for regression models predicting post-treatment depression scores indicated that change in depression was not significantly associated with condition (CES-D:  $B_{\text{CONDITION}} = 1.06, SE = 1.22, 95\% \text{ CI: } .67 \text{ to } 1.56, p = .39$ ). However, depression decreased from baseline to post-treatment regardless of condition (CES-D: Mean Difference:  $-3.10, 95\% \text{ CI} = -4.59 \text{ to } -1.61, t = -4.08, p < .0001$ ). Results of regression estimates predicting psychological symptoms as measured by the GSI (BSI), Deficits in Emotion Regulation as measured by the DERS, and Physical Symptoms as measured by the PILL also indicated that change in these measures was not significantly associated with condition. However, general psychological symptoms decreased from baseline to post-treatment regardless of condition (Global Severity Index, GSI, Mean Difference =  $-.13, 95\% \text{ CI} = -.053 \text{ to } -.20, t = 2.45, p = .02$ ).

With regard to mindfulness facets, those in the mindfulness condition had higher levels of Observe at post-treatment than those in the contact-control condition controlling for baseline levels ( $B_{\text{CONDITION}} = .34, SE = .18, 95\% \text{ CI: } .0079 \text{ to } .69, p = .05$ ), and those in the contact-control condition had higher levels of Nonjudging at post-treatment than those in the mindfulness condition controlling for baseline levels ( $B_{\text{CONDITION}} = -.35, SE = .11, 95\% \text{ CI: } -.57 \text{ to } -.12, p = .0025$ ). See Figures 3.3 and 3.4 for graphs of respective results. Levels of other facets of mindfulness at post-treatment were not significantly

associated with condition when controlling for baseline levels. With regard to psychological well-being, condition was not significantly associated with levels of psychological well-being at post-treatment controlling for baseline levels. However, levels of Personal Growth increased from baseline to post-treatment regardless of condition (Mean Difference = .11, 95% CI = .0021 to .21,  $t = 2.00$ ,  $p = .045$ ).

#### *Longitudinal Outcomes within the Mindfulness Training Condition*

Additional within-group analyses were conducted within the mindfulness condition to investigate 1) the significance of within-condition effects of time at post-treatment, and 2) whether significant changes within the mindfulness condition at post-treatment were maintained at 3-month follow-up (i.e., whether the difference between baseline and 3-month measures were also significant in expected directions). Results indicate that, within the mindfulness condition, IL-6 decreased significantly from baseline at both post-treatment and 3-month follow-up assessments (Mean Difference from baseline to post-treatment =  $-.58$ , 95% CI =  $-.27$  to  $-.98$ ,  $t = -2.38$ ,  $p = .019$ ; Mean Difference from baseline to 3-month follow-up =  $-.75$ , 95% CI =  $-.45$  to  $-1.87$ ,  $t = -1.98$ ,  $p = .054$ ). Identical analyses carried out for TNF- $\alpha$  suggested that, although TNF- $\alpha$  decreased significantly from baseline to post-treatment, the decrease from baseline to the 3-month follow-up assessment was only marginally significant, suggesting that changes in TNF- $\alpha$  may have been less robust (Mean Difference from baseline to post-treatment =  $-.53$ , 95% CI =  $-1.05$  to  $-.41$ ,  $t = -2.38$ ,  $p = .008$ ; Mean Difference from baseline to 3-month follow-up =  $-.45$ , 95% CI =  $-1.48$  to  $.72$ ,  $t = -1.21$ ,  $p = .11$ ). Results for depression scores indicated that the CES-D decreased significantly from baseline to post-treatment (Mean Difference from baseline to post-treatment =  $-2.71$ , 95% CI =  $-3.46$  to  $-1.83$ ,  $t = -2.35$ ,  $p = .018$ ) and from baseline to the 3-month follow-up (Mean Difference from baseline to 3-month follow-up =  $-2.76$ , 95% CI =  $-5.19$  to  $-.32$ ,  $t = -2.25$ ,  $p = .026$ ). Additionally, decreases in GSI, though only marginally significant at post-treatment, were statistically significant at the 3-month follow-up (Mean Difference from baseline to post-treatment =  $-.10$ , 95% CI =  $-.22$  to  $.04$ ,  $t = -1.53$ ,  $p = .13$ ; Mean Difference from baseline to 3-month follow-up =  $-.25$ , 95% CI =  $-.38$  to  $-.13$ ,  $t = -2.51$ ,  $p = .0075$ ). With regard to the Observe facet of the FFMQ, increases were significant at post-treatment (Mean Difference from baseline to post-treatment =  $.29$ , 95% CI =  $.14$  to  $.58$ ,  $t = 2.01$ ,  $p$

= .044) but not at 3-month follow-up (Mean Difference from baseline to 3-month follow-up = .31, 95% CI = -.35 to .98,  $t = .94$ ,  $p = .35$ ). Finally, results indicate that changes in Personal Growth were not significant at either from baseline to post-treatment or from baseline to the 3-month follow-up (Mean Difference from baseline to post-treatment = .13, 95% CI = -.08 to .35,  $t = 1.22$ ,  $p = .22$ ; Mean Difference from baseline to 3-month follow-up = .11, 95% CI = -.25 to .48,  $t = .63$ ,  $p = .53$ ).

### **Aim 2: Potential Mediators of Cytokine Outcomes**

Because there were no effects of mindfulness training on depression, hypothesized meditational models were not considered. However, several post-hoc regression models were fit and combined using PROC MIANALYZE to explore other potential mediators of the effects on salivary cytokines. First, because there were condition effects on changes in the Observe subscale of the FFMQ, this scale was examined as a potential mediator. However, changes in the Observe subscale were not significantly related to cytokines at time 2 controlling for cytokines at time 1 (IL-6:  $B_{OBSERVECHANGE} = -.049$ ,  $SE = .16$ , 95% CI: -.28 to .17,  $p = .76$ ; TNF- $\alpha$ :  $B_{OBSERVECHANGE} = -.074$ ,  $SE = .15$ , 95% CI: -.12 to .20,  $p = .62$ ). Next, we explored condition effects on changes in several health behaviors, including physical activity, caffeine and alcohol consumption. Results indicated that condition was not associated with health behaviors at time 2 controlling for the relevant health behaviors at time 1 (Minutes of Physical Activity per Week:  $B_{CONDITION} = 7.03$ ,  $SE = 3.75$ , 95% CI: -1.59 to 1.12,  $p = .85$ ; Units of Caffeine per Day:  $B_{CONDITION} = -2.09$ ,  $SE = 2.30$ , 95% CI: -5.91 to 1.73,  $p = .93$ ; Units of Alcohol per Week:  $B_{CONDITION} = 2.48$ ,  $SE = 4.16$ , 95% CI: -6.08 to 1.10,  $p = .95$ ). Therefore, condition effects on health behaviors do not appear to be responsible for condition effects on changes in salivary cytokines.

Table 3.1

*Descriptives by Sample*

Variable	Mindfulness Condition	Contact- Control Condition
Age: M (SD)	19.15 (.17)	19.11 (.16)
Grade Level: N (%)		
Freshman	22 (70%)	27 (82%)
Sophomore	6 (20%)	3 (9%)
Junior	3 (10%)	3 (9%)
Race: N (%)		
Caucasian	25 (81%)	30 (91%)
African-American	4 (13%)	1 (3%)
Asian	2 (6%)	2 (6%)
Religious Affiliation: N (%)		
Christian	26 (84%)	25 (76%)
Hindu	1 (4%)	1 (3%)
No Religious Affiliation	2 (6%)	5 (15%)
No Answer	2 (6%)	2 (6%)

*Note.* Standard Deviations and Within-group Percentages in Parentheses



Table 3.2

*Means and Standard Deviations for Outcome Variables at Baseline and Post-treatment by Condition*

Variable	Full Sample		Mindfulness Condition		Contact-Control Condition	
	PRE M (SD)	POST M (SD)	PRE M (SD)	POST M (SD)	PRE M (SD)	POST M (SD)
IL-6	3.58 (4.55)	3.14 (5.36)	3.50 (5.14)	2.20 (2.24)	3.66 (4.01)	4.04 (7.14)
TNF- $\alpha$	2.21 (2.95)	1.64 (1.68)	2.13 (3.76)	1.33 (1.42)	2.29 (1.93)	1.94 (1.87)
CES-D total	12.53 <sup>a</sup> (6.25)	9.44 <sup>b</sup> (5.64)	13.26 <sup>a</sup> (5.67)	10.30 <sup>b</sup> (5.89)	11.85 <sup>a</sup> (6.76)	8.63 <sup>b</sup> (5.36)
GSI (BSI)	.51 <sup>a</sup> (.30)	.40 <sup>b</sup> (.25)	.53 (.31)	.43 (.24)	.48 <sup>a</sup> (.30)	.37 <sup>b</sup> (.26)
PILL total	1.81 (.36)	1.75 (.39)	1.78 (.33)	1.73 (.34)	1.84 (.38)	1.77 (.43)
Lack of Emotional Awareness (DERS)	2.58 (.72)	2.47 (.70)	2.56 (.82)	2.44 (.71)	2.60 (.64)	2.50 (.71)
Lack of Emotional Clarity (DERS)	2.14 (.63)	2.20 (.60)	2.26 (.76)	2.27 (.64)	2.02 (.45)	2.12 (.55)
Lack of Acceptance of Emotional Responses (DERS)	1.90 (.70)	1.94 (.80)	1.84 (.58)	2.00 (.86)	1.95 (.80)	1.88 (.74)
Limited Access to Strategies (DERS)	1.73 (.47)	1.71 (.53)	1.77 (.45)	1.68 (.48)	1.68 (.50)	1.75 (.58)

Table 3.2 (Continued)

Lack of Impulse Control (DERS)	1.57 (.38)	1.53 (.45)	1.54 (.37)	1.45 (.41)	1.59 (.39)	1.60 (.48)
Difficulties with Goal-Directed Behavior (DERS)	2.87 (.77)	2.81 (.83)	2.91 (.86)	2.90 (.84)	2.82 (.70)	2.73 (.82)
Observe (FFMQ)	3.44 (.83)	3.49 (.82)	3.32 <sup>a</sup> (.83)	3.57 <sup>b</sup> (.75)	3.54 (.82)	3.41 (.89)
Describe (FFMQ)	3.40 (.67)	3.39 (.78)	3.29 (.80)	3.35 (.83)	3.46 (.53)	3.43 (.75)
Nonjudging (FFMQ)	3.81 (.68)	3.88 (.64)	3.80 (.69)	3.70 (.65)	3.81 <sup>a</sup> (.67)	4.04 <sup>b</sup> (.60)
Nonreactivity (FFMQ)	2.88 (.60)	2.89 (.50)	2.86 (.67)	2.90 (.48)	2.89 (.53)	2.88 (.52)
Acting with Awareness (FFMQ)	3.58 (.65)	3.47 (.69)	3.50 (.66)	3.38 (.74)	3.66 (.64)	3.55 (.63)
Autonomy (PWB)	4.18 (.72)	4.16 (.73)	4.17 (.74)	4.18 (.78)	4.20 (.72)	4.14 (.69)
Environmental Mastery (PWB)	4.27 (.69)	4.40 (.61)	4.21 (.73)	4.40 (.57)	4.33 (.67)	4.39 (.66)
Self-Acceptance (PWB)	4.71 (.66)	4.72 (.68)	4.79 (.64)	4.90 (.57)	4.64 (.68)	4.55 (.75)
Positive Relations (PWB)	4.67 (.79)	4.75 (.78)	4.56 (.82)	4.64 (.80)	4.78 (.76)	4.85 (.77)
Purpose in Life (PWB)	4.70 (.55)	4.69 (.60)	4.75 (.58)	4.76 (.65)	4.66 (.52)	4.63 (.56)
Personal Growth (PWB)	4.91 <sup>a</sup> (.59)	5.02 <sup>b</sup> (.67)	5.01 (.62)	5.04 (.68)	4.81 <sup>a</sup> (.56)	5.00 <sup>b</sup> (.67)

Note: Differing superscripts indicate significant differences between baseline and post-treatment values ( $p < .05$ ).

Table 3.3

*Estimates for Poisson Regression Models Predicting IL-6 and TNF- $\alpha$  Using PROC MIAnalyze*

Variable	Estimate	95% CI for Estimate
<u>Interleukin-6</u>		
Oral Contraceptive Use	-.70*	-1.06 to -.34
Time 1 IL-6	.30*	.18 to .41
MBSR Condition	-.60*	-.95 to -.26
<u>Tumor Necrosis Factor-<math>\alpha</math></u>		
Oral Contraceptive Use	-.66*	-1.14 to -.17
Body Mass Index	.12	-.10 to .35
Time 1 TNF- $\alpha$	.30*	.15 to .44
MBSR Condition	-.37*	-.86 to -.12

*Note: \* $p < .01$*

Figure 3.1

*Effect of Condition on Change in IL-6 Pre-to-Post-Treatment*

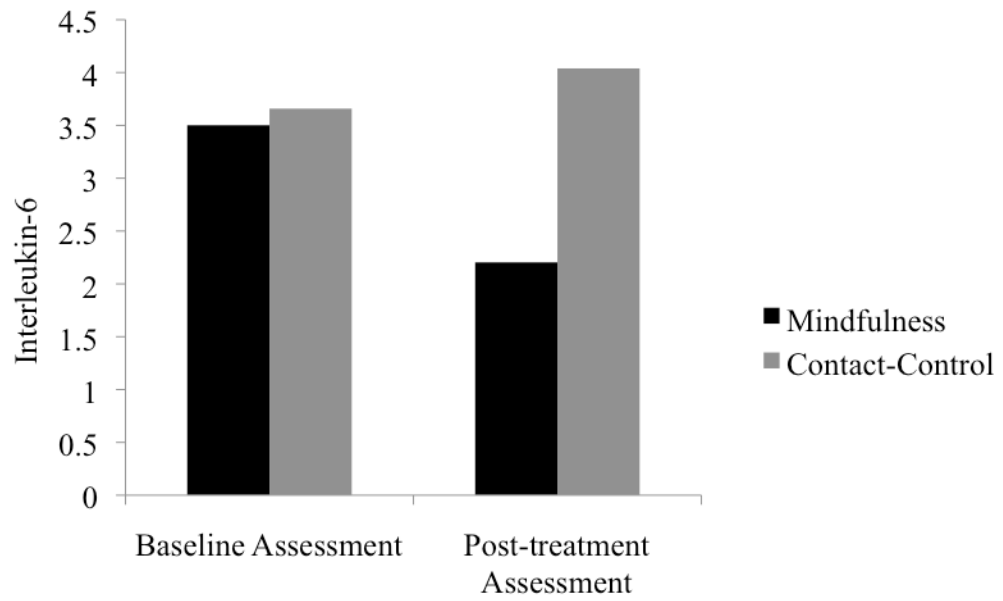


Figure 3.2

*Effect of Condition on Change in TNF- $\alpha$  Pre-to-Post-Treatment*

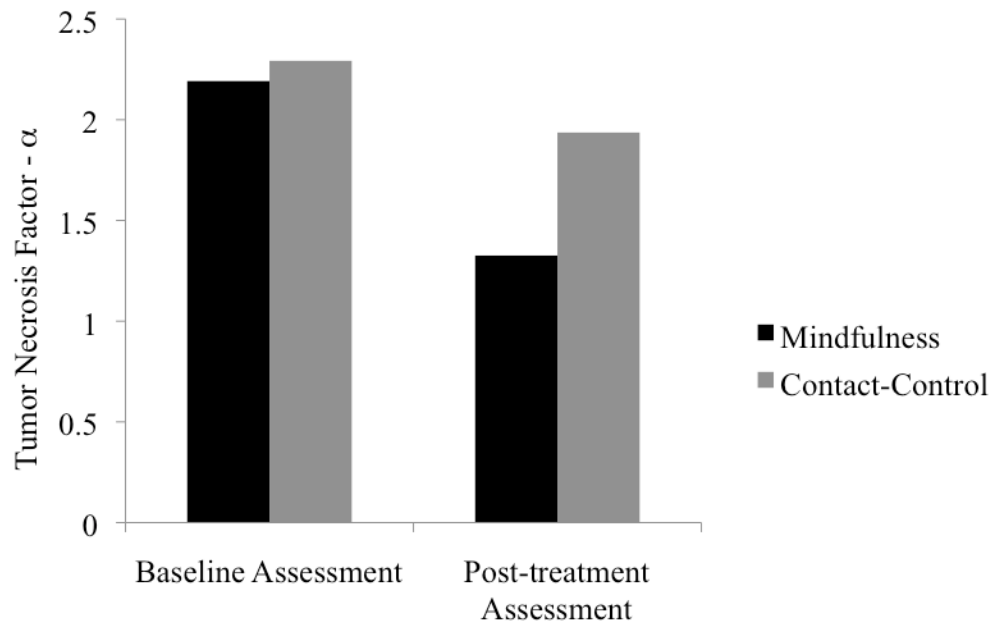


Figure 3.3

*Effect of Condition on Change in Observe Pre-to-Post-Treatment*

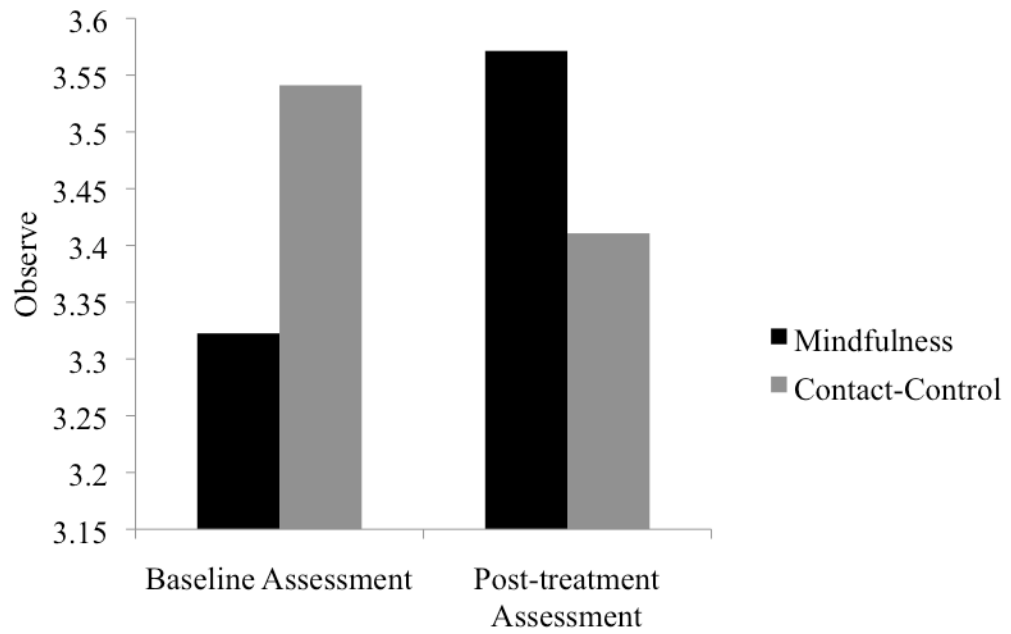
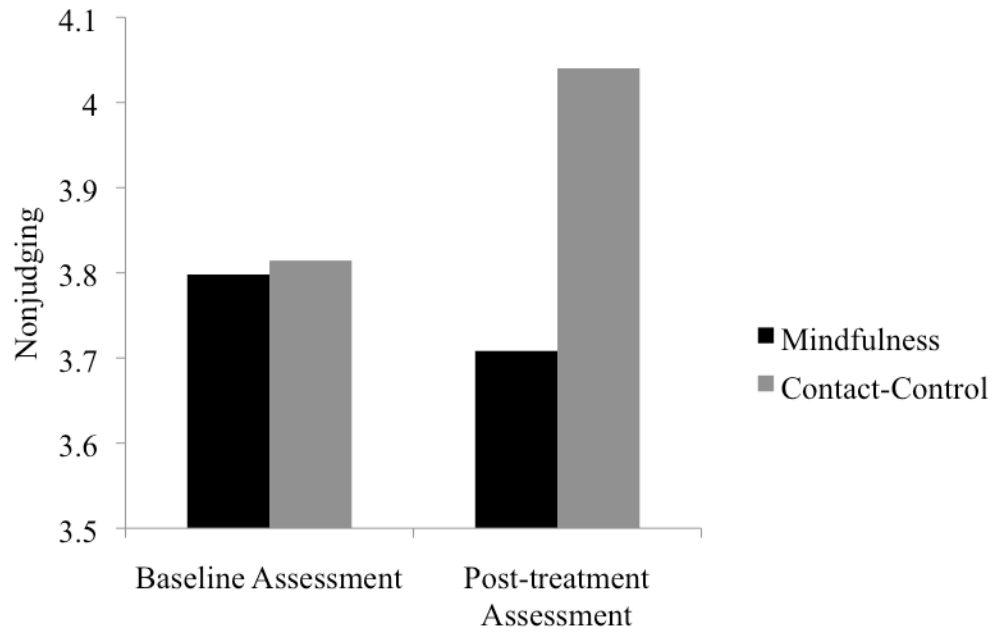


Figure 3.4

*Effect of Condition on Change in Nonjudging Pre-to-Post-Treatment*



## Section Four: Discussion

The present study investigated whether a short-term mindfulness-based intervention could reduce both immunological and psychological features of depression in a sample of moderately depressed college women. The first hypothesis was that participation in the mindfulness group would be associated with post-treatment reductions in pro-inflammatory immune correlates of depression: IL-6 and TNF- $\alpha$ . This hypothesis was supported. Compared to a contact-control group, participants in the mindfulness intervention showed significant reductions in these two immune variables. The second hypothesis was that participation in the mindfulness group also would be associated with changes in several self-reported psychological variables, including depression, general distress, well-being, emotion regulation, and mindfulness. This hypothesis was not supported. Although improvements in several psychological variables were observed, very few between-group differences emerged. The Observe facet of the FFMQ was shown to significantly increase in the mindfulness group only, while the Nonjudging facet increased only in the contact-control group. The third hypothesis was that, for participants in the mindfulness intervention, improvements in immune and psychological health variables would remain stable over the 3-month follow-up period. This hypothesis was partially supported. Within the mindfulness group, reductions in both pro-inflammatory cytokines were sustained at a 3-month follow-up assessment. However, because minimal differences in self-reported psychological variables emerged pre-post-treatment, there were very few treatment effects in this domain to monitor at the 3-month follow-up. While increases in the Observe facet were significant from baseline to post-treatment in the mindfulness condition, these changes were not maintained; however, reductions in psychological distress as measured by the Global Severity Index (GSI) were significant from baseline to post-treatment and maintained at 3-month follow-up in the mindfulness condition. The fourth hypothesis was that self-reported depression at post-treatment would mediate the relationship between participation in mindfulness training and changes in IL-6 and TNF- $\alpha$ . This hypothesis was not tested because, contrary to expectations, the relation between participation in mindfulness training and change in depression was not significant.



Results for IL-6 and TNF- $\alpha$  are consistent with other investigations examining the effects of short-term (e.g., Tang et al., 2007) or full-length (e.g., Carlson et al., 2007) mindfulness-based interventions on immunity. For example, significant reductions in pro-inflammatory cytokines have been observed in cancer patients enrolled in 8-week mindfulness courses (Carlson et al. 2003; Witek-Janusek et al., 2008) and these results were sustained at a one-year follow-up (Carlson et al., 2007). Similar to these findings, we observed significant reductions in cytokines pre-post treatment for the mindfulness training group only and found that these improvements were maintained at 3-months following treatment completion. However, this investigation is the first to demonstrate such effects in a dysphoric yet immunocompetent sample and the first to include both a control group and a post-treatment follow-up assessment. Furthermore, we provide evidence that such reductions can occur with relatively brief training in mindfulness practices. The current findings support the notion that mindfulness training may promote immune responses that contribute to physical health (e.g., less inflammation). It underscores the importance of examining such processes in future samples with depressive disorders because doing so may illuminate the mechanisms by which mindfulness contributes to improvements in psychological health.

The failure of the current study to find changes related to mindfulness training in self-reported psychological variables is inconsistent with overwhelming evidence suggesting that mindfulness training has direct benefits on many of the constructs measured in this study (see Keng et al., 2011 for a review). Several factors may account for these findings. One possibility is the low level of psychological distress in the sample. Participants were recruited if they reported moderate levels of depressive symptoms (scores of 16-24 on the CES-D) at the initial screening; however, mean levels of depression had fallen to 12.53 (indicating minimal to mild symptoms) by the first session, suggesting that the initial screening scores may have been temporary elevations related to situational stress, rather than indicators of sustained depressive symptoms. As evidence for this point, participants showed further decreases in depressive symptoms from pre- to post-treatment. These changes are likely attributable to further regression to the mean as the semester progressed. A second possibility is that the “dose” of mindfulness training in the current study was not strong enough to effect changes in self-report measures that go

beyond regression to the mean. Perhaps if participants had been provided with additional training sessions (i.e., more than 4) or longer training sessions (i.e., more than 50 minutes), robust between-group differences in psychological variables may have emerged. Several previous studies with comparable training lengths have reported reductions in psychological symptoms or improvements in well-being in student samples (Tang et al., 2007; Sauer et al., under review), while others have not (Pace et al., 2009). Thus, this is an important area for further inquiry. A third potential factor is the non-treatment seeking status of the sample. Although they had reported moderate levels of depression at the initial screening, they were not recruited from a help-seeking population, but instead completed the study to meet a research participation requirement. They engaged in very little home practice and may have had low levels of motivation to learn mindfulness skills. Although a recent review (Carmody & Baer, 2009) found no significant relationship between treatment duration and psychological symptom reduction, suggesting that short treatment formats may be as effective as longer ones, most of the reviewed studies used clinical populations or nonclinical samples seeking help for stress reduction.

Despite its nonsignificant relationships with changes in self-reported psychological functioning, the short-term mindfulness training provided in this study was sufficient to lead to significant reductions in the two immune variables studied. It is therefore important to consider how these immune changes occurred. It appears that they are not accounted for by the self-report variables measured here. However, it is entirely possible that we did not assess variables that could directly account for the significant reductions in salivary cytokines. One particularly likely candidate is rumination. Rumination is a transdiagnostic process, meaning that it is present in a multitude of psychological disorders. Although definitions vary, it can be thought of as repetitively focusing on one's emotional experience, as well as the causes, consequences, and implications of it (Aldao, Nolen-Hoeksema, & Schweizer, 2010). Individuals who ruminate believe that it will help them solve problems or improve mood, however, investigations show that rumination interferes with adaptive problem-solving (Aldao, Nolen-Hoeksema, & Schweizer, 2010) and perpetuates negative mood (Thomsen, Mehlsen, Christensen, & Zachariae, 2003). Mindfulness is thought to reduce rumination

by teaching participants to defuse or decenter from thoughts via nonreactive observation or to focus on other present-moment stimuli, such as the breath or bodily sensations (Baer, 2007; Uebelacker et al., 2010). Investigations have shown that mindfulness training is efficacious in reducing rumination in samples experiencing depressive or general psychological symptoms (Kingston et al., 2007; Heeren & Philippot, 2011). Additionally, Sauer, Walsh, Lykins and Eisenlohr-Moul (under review) showed that even short-term, 3-session training in separate mindfulness practices led to significant reductions in rumination. In fact, rumination was the only symptom that all mindfulness practices significantly and robustly reduced. In regard to physiological correlates, there is prospective evidence suggesting that rumination is associated with self-reported somatic complaints (e.g., Thomsen et al., 2004a). Rumination is also linked to increases in morning levels of cortisol (Schlotz et al., 2004), elevated leukocytes (Thomsen et al., 2004b), and reductions in T-lymphocytes (Denson et al., 2010). These findings suggest that rumination may have negative effects on one's physical health. Because mindfulness training is associated with reductions in ruminative tendencies, it is entirely plausible that such changes may have mediated effects on cytokine levels in the present study.

### *Limitations*

The present study has several limitations worth noting. First, as mentioned earlier, participants with moderate depressive symptoms were recruited with a widely used screening measure, the Center for Epidemiological Studies Depression Scale (CES-D; Radloff, 1977). Although scores within the "moderate" depression range (i.e., 16-24) are thought to indicate concern for depressive disorders, the presence of specific depressive disorders was not thoroughly assessed, and many individuals' scores had decreased from the initial screening session to the baseline assessment. Although a structured interview was used during the phone screening, we did not require that participants meet criteria for major depressive episodes or dysthymia to be admitted into the study. The structured interview was only utilized to rule out individuals who reported the presence of certain diagnoses (e.g., PTSD) or psychological features (e.g., suicidality). Future investigations examining the effects of mindfulness training on depression would benefit from using structured clinical interviews, such as the SCID-I, to recruit participants meeting criteria for DSM-IV depressive disorders. If using a short

screening measure of depression, the Beck Depression Inventory (BDI) may be preferred to the CES-D because it has been shown to have fewer false positives in regard to adolescent or young adult samples (Roberts et al., 1991). If the CES-D is utilized, researchers should consider using higher cut scores when selecting for depressed young adults (Lawrence et al., 2006).

Second, the current study did not document longitudinal changes in the contact-control group due to a requirement by the IRB that mindfulness training should be offered to control participants the semester following the 6-week post-treatment assessment (all declined). Future studies should assess both intervention and control groups at all three time points. This will allow researchers to more thoroughly assess whether maintained effects are the result of treatment or time.

Finally, the current study did not offer a full-length, empirically supported mindfulness training program, such as MBSR or MBCT, to participants with depressive symptoms. These programs, which typically include 8 weekly 2-hour sessions, have been shown to have significant therapeutic effects on symptoms and well-being (e.g., Keng et al., 2011) and to reduce the risk of depressive relapse (e.g., Piet & Hougaard). It is quite possible that additional sessions or treatment components not offered in the current study may be necessary for cultivating meaningful increases in mindfulness and emotion regulation and for increasing awareness of emotional and cognitive processes so that participants can accurately reflect and report on changes that occur over the course of treatment.

Finally, although the present study included a longer follow-up than is typical for this literature, an even longer follow-up would have been useful for examining whether the change in immune variables seen at post-treatment and 3-month follow-up was associated with protection against future episodes of depressive symptoms.

### *Conclusion*

Despite the lack of condition effects on changes in numerous psychological variables, the current study showed that only mindfulness training led to improvements in immune functioning. Specifically, levels of salivary pro-inflammatory cytokines were reduced at post-treatment in the mindfulness condition, and these effects were maintained at a 3-month follow-up assessment. This is noteworthy because it demonstrates that even

short-term training in mindfulness could have important implications for physical and psychological health. There is some evidence to suggest that higher baseline levels of pro-inflammatory cytokines in healthy individuals are risk factors for the development of disorders such as depression (Wichers et al., 2006). Although the directionality of the inflammation-depression relation is complex (Howren et al., 2009), it could be that reductions in pro-inflammatory cytokines of the kind seen in the mindfulness condition are capable of preventing the development of future episodes of depression.

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