

**The Effects of Mindfulness Meditation on Psychoneuroendocrinoimmunology in**

**Nursing Students**

Study Protocol

Date: July 30, 2019

NCT number: Not yet issued

## **Introduction**

Stress has been recognized worldwide as a prevalent health issue in nursing education (1). Like nursing students in other countries, nursing students in Jordan have reported moderate to high levels of stress, sometimes higher than the levels reported by students from other disciplines (2, 3). Although acute stress works to sustain human hemostasis, repeated exposure to stress can influence students' health, academic performance, attrition, and ability to care for patients and themselves (4).

Previous studies have shown that stress can negatively impact the body. Specifically, the immune system can be negatively impacted by the activation of the hypothalamic-pituitary-adrenal (HPA) system (e.g. cortisol) (5). Because of stress, the hypothalamus releases a corticotrophin-releasing factor (CRF) that stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior areas of the pituitary gland. ACTH stimulates the adrenal cortex to secrete cortisol, described as a stress hormone that mainly affects metabolic processes, and an elevated cortisol level suppresses the immune system and inflammatory functions (5). Further, an elevated cortisol level increases the release of pro-inflammatory factors such as C-reactive protein (CRP), which is the most widely used proinflammatory biomarker in clinical practice and which has been found to be associated with a variety of chronic illnesses such as major depression, coronary artery diseases, cancer, and diabetes mellitus (6, 7).

It has been reported that when dealing with stress, Jordanian nursing students mostly use inefficient, harmful coping methods such as avoidance and smoking. These methods have short-lived benefits but are harmful in the long run, resulting in a variety of physical and psychological health problems (8, 9). Therefore, nursing students in Jordan may need to learn to use more effective coping strategies.

There are several potentially beneficial stress-reduction methods which have been found to help students worldwide cope effectively with stress and its adverse health consequences (10). Mind-body therapies are being more widely used in Jordan, as they are easily accessible, easy to learn, and inexpensive (11). Specifically, mindfulness meditation is a mind-body therapy that has been gaining increasing attention in the recent literature and has demonstrated promising results across different populations and settings (12, 13). The target groups for mindfulness meditation include people with chronic physical pain, illnesses such as cancer, or mental illnesses, such as stress, anxiety, depression, or burnout (12). In addition, this mind-body therapy has been used among non-clinical populations, including students, therapists, and prisoners (14).

Whilst mindfulness meditation has been shown by a growing body of evidence to improve several health and psychological well-being measures in university students, its mechanism of action and how it improves stress and its related health responses remain debated in the literature. Garland et al. (2009) proposed a hypothetical causal model that argues for the role of mindfulness meditation in positive reappraisal coping, explaining that attending repeatedly in a mindful way evokes a transitory metacognitive state of mindfulness that remains for as long as that form of attention is sustained. With repeated practices, individuals, including students, can cultivate their predisposition to be mindful in daily life, which is known as trait mindfulness (15). Trait mindfulness is described as the cognitive ability to non-judgmentally observe sensations, thoughts, emotions, and the environment while simultaneously encouraging openness, curiosity, and acceptance (16). Previous studies among university students have supported this theoretical claim. A recent randomized controlled trial showed that four weekly sessions of mindfulness meditation improved trait mindfulness among university students and staff (N=561) (17), and another recent RCT

showed that three daily sessions of mindfulness meditation significantly improved state mindfulness among university students (N=40) (18).

Based on the mindful coping model (15), positive reappraisal is a cognitive coping strategy that enables people to adapt successfully to perceived stress, which is defined as the degree to which events are appraised as stressful and uncontrollable (19). For example, a student who perceives a given event or situation, such as an exam, as stressful (e.g. risk of threat, harm, or loss) and exceeding their abilities may practice mindfulness meditation to induce an adaptive response by decentring from this stress appraisal into a mode of mindfulness. In turn, this mode allows the student to pay attention to the dynamic process of consciousness itself rather than its contents, which enhances attentional flexibility and raises awareness. Based on this broadened, metacognitive awareness, the student can then reappraise the given event in a positive way by giving it a new meaning, which may evolve either through a conscious process of reflection or a more automatic process. The reappraisal of the event then leads to positive emotions such as compassion, trust, and confidence that ultimately decrease stress and its related physiological reactions, such as cortisol and CRP (15).

Previous studies have also supported the claims of this model regarding the effects of mindfulness meditation on perceived stress among university students. A previous RCT showed that 8 weeks of mindfulness meditation reduced study-related stress among university students (20). In addition to long-term interventions, RCTs using brief mindfulness meditation, with sessions ranging between 3 daily sessions to 4 weekly sessions, showed that these brief mindfulness meditation interventions decreased perceived stress among university students [21, 22, 23, 24].

In addition to its psychological effects, mindfulness meditation has been found to improve physiological indices of stress. It is hypothesized that mindfulness meditation

buffers stress reactivity, resulting in decreased cortisol responses and proinflammatory markers such as CRP. In a systematic review of 45 RCTs (25), the authors identified strong evidence of mindfulness meditation leading to reductions in serum cortisol and C-reactive protein. Similarly, another systematic review of RCTs concluded that mindfulness meditation has been found to reduce pro-inflammatory cytokines, including CRP, which is associated with the reduction of perceived stress and cortisol (26).

Although an increasing number of studies have demonstrated the effectiveness of mindfulness meditation in improving wellbeing and reducing stress among ill and healthy populations such as university students, these studies have been conducted only in western countries. Therefore, any generalization of these study findings to other populations of different cultures is limited due to these cultures' differing belief systems and self-care practices. Also, there have been in these previous studies variations in the operationalization and standardized delivery of mindfulness meditation, limiting the generalizability of the findings related to the distinct effects of this therapy on stress and health. In Jordan, there is a lack of evidence related to the effectiveness of mindfulness meditation on stress and its physiological indices in Jordanian people generally and nursing students specifically. Thus, this study aimed to examine the effects of a standardized version of mindfulness meditation on trait mindfulness, perceived stress, serum cortisol, and serum CRP in Jordanian nursing students.

The current study is the first to report findings related to the effects of mindfulness meditation on psychoneuroendocrinoimmunology among the target population. Practically, the findings of this study may guide nursing faculties in adopting mindfulness meditation in nursing colleges. Theoretically, the current study also extends the body of nursing knowledge and fills a gap in the literature regarding the effects of mindfulness meditation on psychoneuroendocrinoimmunology in nursing students outside the United States and Europe.

## **Methods**

### **Design and Participants**

The study used randomized controlled design (pre-posttest, two-group, randomized, blinded design). The study sample included undergraduate nursing students who were selected using convenience sampling from Jordan University of Science and Technology (JUST). Students were included if they were aged 18 years or over and taking a clinical course. Meanwhile, students were excluded if they were practicing any type of relaxation techniques or taking hypnotics, sedatives, anxiolytics, anti-depressants, or anti-hypertensive drugs.

### **Sample Size Calculation**

G\* Power software 3.1 was used to calculate the required sample size, given a MANOVA test, an alpha of .05, power of 0.95, number of dependent variables of 4, number of groups of 2, and effect size of 0.25 (18). A sample size of 80 was calculated, and an attrition rate of 35% was added based on a previous study with variables similar to those of the present study (27). Therefore, this resulted in a final total sample size of 108 participants.

### **Instruments**

The study data were collected using self-reported and physical measures. For the self-reported variables, a self-report questionnaire was used. The first part of the questionnaire included questions related to the behaviors and demographic characteristics of the participants, including age, religion, gender, marital status, daily consumption of cigarettes, general health status, nutritional status, coping behaviors, and sleeping hours and quality. Meanwhile, the second part included scales used to measure trait mindfulness and perceived stress. As for the physical variables, the enzyme-linked immunosorbent assay (ELISA) procedure was used to measure serum biomarkers such as serum cortisol and serum CRP.

**Trait Mindfulness.** Trait mindfulness was measured using the Arabic version of the Mindful Attention Awareness Scale (MAAS) (28), which is a one-dimensional scale that includes 15 items measured on a six-point Likert-type scale ranging from 1 (almost always) to 6 (almost never). The total possible score ranges between 15 and 90, with higher scores indicating higher levels of trait mindfulness. The original scale has been shown to have strong psychometric properties when validated among college students (28). Further, the Arabic version of this scale has been shown to have excellent internal consistency and convergent validity for use among Arab populations (29). In the current study, the Cronbach's alpha for the Arabic version of the MAAS was .79, indicating good internal consistency.

**Perceived Stress.** The Perceived Stress Scale (PSS) (30) is used to measure the degree to which situations in one's life are appraised as stressful (unpredictable, uncontrollable, and overloaded). The scale comprises 10 items which are measured on a 5-point Likert scale (0=never, 4= very often) and which are relatively free of content specific to any subpopulation group. The total possible score ranges from 0 to 40, with higher scores indicating higher levels of perceived stress (30). The PSS has been validated for use among college students, with internal consistency coefficients ranging from .84 to .86 and a test-retest reliability of .85 (30). The Arabic version of the PSS, which was used in the present study, has been shown to have adequate reliability and validity and is considered a suitable instrument for assessing perceived stress among Arab populations (31). In the current study, the Cronbach's alpha for the Arabic version of the PSS was .81, indicating good internal consistency.

### **Biomedical Markers.**

The ELISA method has been found to be accurate, sensitive, specific, and precise in terms of determining the plasma concentration of cortisol and proinflammatory markers (32). In the current study, the ELISA kit and protocols were used to analyze the blood sample for the serum cortisol (CEA462Ge) and serum CRP (Cat no: SEA821Hu) . Three research

assistants with at least 10 years of experience collected 10 mL blood samples via venipuncture both at baseline and next day morning after the last session, between 8:30-9:30 AM. The results were read using a SYNERGY (HTZ) multi-mode reader, connected to a computer which showed each reading value. The participants were asked to avoid caffeine consumption on the days of data collection. To decrease venipuncture-related pain during blood sampling, the EMLA cream was used, as it has been found effective in decreasing venipuncture-related pain among different populations (33).

### **Interventions**

In the current study, the ABC standardized version of mindfulness meditation was used, which includes 5 30-minute weekly sessions of mindfulness meditation and which has been found effective in improving stress and its responses (34). An additional 3-hour educational workshop about the intervention was provided before the actual training sessions to enhance the participants' understanding of the intervention. In the initial educational session, the principal investigator (PI) held a PowerPoint presentation for the participants in each experimental group, which included an explanation of the intervention rationale and procedures and a demonstration of the entire intervention protocol. The PI is an experienced practitioner who received stress-management training at the Psychology Department at Kent State University over 7 years ago and who has, since then, been practicing stress-management techniques, especially those used in the current study, on a daily basis. The participants were asked to briefly practice the intervention, and their performance was then evaluated by the PI to make sure that the intervention had been conducted correctly. The PI gave the participants the opportunity to ask questions during the educational session.

During the actual training sessions, the intervention was introduced to the participants in the experimental groups by the PI based on Smith's (2005) guidelines and protocols. The participants were divided into 6 groups, with 10 to 11 participants in each group. The



subgroups met separately in different classrooms, where the interventions were conducted, and provided with identical instructions. Each participant sat in a hard chair behind a desk. The classrooms had tiled floors, and the lights were dimmed during the relaxation phases and turned up when the participants were completing the study questionnaires. A trained research assistant with a master's degree in psychiatric health nursing randomly selected sessions to evaluate the delivery of the interventions using checklists produced according to Smith's protocols.

Smith's version of the mindfulness meditation intervention was used in the current study. The participants were asked to sit upright in a comfortable position, place their feet on the floor, and quietly observe and reflect on internal and external stimuli such as breathing, thought, feeling, physical sensation, and sound, without reactions, judgments, or evaluations. They were asked to quietly attend to, note, and let go of all internal and external stimuli. They were reminded that they should not try to think about, push away, or do anything with these stimuli, and that they should not focus on figuring out the connections between the stimuli. They were asked to simply let each stimulus come and go and wait for the next stimulus. Finally, the participants were told that they should not concern themselves with distractions and that if faced with a distraction, they should note it as yet another passing stimulus (e.g., "Ah, a distraction... how interesting") (34).

### **Control Group**

Each of the control group subgroups had 10 participants. The participants were instructed to sit with their eyes closed and relax during the intervention sessions, in order to control for the nonspecific effects of trainer interaction, social interaction, attention, environment, time, and closed eyes (35). The timings of the control group interventions were similar to those of the experimental groups, whereby if a given experimental group

intervention lasted for 30 minutes, the control group participants would be asked to sit with their eyes closed and relax for 30 minutes also.

## **Procedure**

First, approval from the institutional review board at JUST was obtained. After obtaining permission from the dean of the nursing college at JUST and the students' instructors, the PI visited the students in their classrooms. After the students' lecture had finished, the PI made an announcement explaining the study title and objectives and asked any students who were interested in participating to contact him either on the phone number assigned for the purposes of the study or via email. The PI arranged an initial meeting to meet with students who were interested in participating, which was to be held on another day in a private room at the university. At this meeting, the PI informed the students of the study objectives, risks, and benefits, in addition to answering the students' questions and assuring them that all collected data would be kept confidential. The students were also assured that they had the full right to refuse or discontinue participation at any time and that such refusal or discontinuation would not affect their academic achievement in any way. At the same meeting, informed consent was obtained from participants who agreed to participate and who met the eligibility criteria. After that, the participants were randomly assigned equally to the study groups using a computerized random numbers procedure carried out by a research assistant who was not involved in any other parts of the study. Finally, the dates and times of the sessions were determined.

In the 2-hour educational session, baseline measurements of the study variables were taken. Then, the PI delivered a PowerPoint presentation for the participants in each subgroup, as previously explained. At the end of the workshop, to avoid experimental contamination, the participants in each experimental group were asked not to share any information related to the intervention with the participants in the control group.

The actual training sessions were introduced to the participants in the experimental groups by the PI based on Smith's (2005) guidelines and protocols. Based on the ABC Relaxation Theory (Smith, 1999, 2002, 2005), at least two, and preferably five, weekly sessions of actual mind-body training should be provided to evoke relaxation and optimize health (36, 37). The sessions were held in a private, quiet, comfortable, and spacious room at the university. As previously mentioned, the dependent variables were measured twice, at baseline and at the end of the intervention (i.e., In the next morning after the 5<sup>th</sup> session).

Various strategies were followed to decrease measurement errors that could potentially be affected by variations in the data collection procedure. For example, filling out the questionnaire may be stressful for some subjects, hence influencing the objective measures. Therefore, the self-report measures were completed after the physical measures were taken. Also, all of the study measures in the intervention and control groups were taken in similar conditions, including similar room temperature and environment. In addition, quiet environments were maintained, with a "Do Not Disturb" sign placed on the doors of the rooms during the interventions and data collection. The data (questionnaire and blood samples) were collected by three research assistants with at least 10 years nursing experiences who were not involved in any other parts of the study.

### **Statistical Analysis**

Statistical analysis was conducted using SPSS software (version 25). First, the data were examined for outliers, missing data, and violations of statistical tests assumptions and managed as appropriate. Descriptive statistics were used to describe the sample. The sample and study variables were described by measures of central tendency and dispersion appropriate to the level of measurement. Initial independent t-tests and Chi-squared tests were conducted to ensure that the randomization across the covariates was successful, and one-way MANOVA was used to test the study hypotheses. Post hoc one-way ANOVAs were

run to examine if there were significant differences in each of the dependent variables between the study groups. Due to multiple testing, the p-value was adjusted by dividing the p value of .05 by 4 (i.e., the number of dependent variables), with a level of .0125 considered as the level of significance for the ANOVA tests.

## References

1. Foster K, Roche M, Giandinoto JA, Platania-Phung C, Furness T. Mental health matters: A cross-sectional study of mental health nurses' health-related quality of life and work-related stressors. *International journal of mental health nursing*. 2021 Jun;30(3):624-34.
2. Al-Zayyat AS, Al-Gamal E. Perceived stress and coping strategies among Jordanian nursing students during clinical practice in psychiatric/mental health courses. *International journal of mental health nursing*. 2014 Aug;23(4):326-35.
3. Labrague LJ, McEnroe-Petitte DM, De Los Santos JAA, Edet O. Examining stress perceptions and coping strategies among Saudi nursing students: a systematic review. *Nurse Educ Today*. 2018;65:192–200. doi:10.1016/j.nedt.2018.03.012
4. Chaabane S, Chaabna K, Bhagat S, Abraham A, Doraiswamy S, Mamtani R, Cheema S. Perceived stress, stressors, and coping strategies among nursing students in the Middle East and North Africa: an overview of systematic reviews. *Systematic reviews*. 2021 Dec;10(1):1-7.
5. Kinlein, Scott A., and Ilia N. Karatsoreos. "The hypothalamic-pituitary-adrenal axis as a substrate for stress resilience: Interactions with the circadian clock." *Frontiers in neuroendocrinology* 56 (2020): 100819.
6. Knight, Erik L., et al. "Perceived stress is linked to heightened biomarkers of inflammation via diurnal cortisol in a national sample of adults." *Brain, Behavior, and Immunity* 93 (2021): 206-213.
7. Markozannes, Georgios, et al. "Global assessment of C-reactive protein and health-related outcomes: an umbrella review of evidence from observational studies and Mendelian randomization studies." *European Journal of Epidemiology* 36.1 (2021): 11-36.

8. Khader YS, Alsadi AA. Smoking habits among university students in Jordan: prevalence and associated factors. *EMHJ-Eastern Mediterranean Health Journal*, 14 (4), 897-904, 2008. 2008.
9. Khater W, Akhu-Zaheya L, Shaban I. Sources of stress and coping behaviours in clinical practice among baccalaureate nursing students. *International Journal of Humanities and Social Science*. 2014 Apr;4(6):194-202.
10. Amanvermez Y, Rahmadiana M, Karyotaki E, de Wit L, Ebert DD, Kessler RC, Cuijpers P. Stress management interventions for college students: A systematic review and meta-analysis. *Clinical Psychology: Science and Practice*. 2021 Aug 12.
11. Yanyu J, Xi Y, Huiqi T, Bangjiang F, Bin L, Yabin G, Xin M, Junhua Z, Zhitao Y, Xiaoyun C, Changsheng D. Meditation-based interventions might be helpful for coping with the Coronavirus disease 2019 (COVID-19).
12. Zhang D, Lee EK, Mak EC, Ho CY, Wong SY. Mindfulness-based interventions: an overall review. *British Medical Bulletin*. 2021 Jun;138(1):41-57.
13. M. Virgili, Mindfulness-based interventions reduce psychological distress in working adults: a meta-analysis of intervention studies, *Mindfulness* 6 (2) (2015) 326–337, <https://doi.org/10.1007/s12671-013-0264-0>.
14. De Vibe M, Solhaug I, Tyssen R, Friberg O, Rosenvinge JH, Sørli T, et al. Mindfulness training for stress management: a randomised controlled study of medical and psychology students. *BMC Med Educ*. 2013;13.
15. Garland E, Gaylord S, Park J. The role of mindfulness in positive reappraisal. *Explore*. 2009 Jan 1;5(1):37-44.
16. Kabat-Zinn, Mindfulness-based interventions in context: past, present, and future, *Clin. Psychol. Sci. Pract.* 10 (2) (2003) 144–156, <https://doi.org/10.1093/clipsy/bpg016>

17. Lahtinen O, Aaltonen J, Kaakinen J, Franklin L, Hyönä J. The effects of app-based mindfulness practice on the well-being of university students and staff. *Current Psychology*. 2021 May 1:1-0.
18. de Sousa GM, de Lima-Araújo GL, de Araújo DB, de Sousa MB. Brief mindfulness-based training and mindfulness trait attenuate psychological stress in university students: a randomized controlled trial. *BMC psychology*. 2021 Dec;9(1):1-4.
19. Lazarus RS, Folkman S. *Stress, appraisal, and coping*. Springer publishing company; 1984 Mar 15
20. Galante J, Dufour G, Vainre M, Wagner AP, Stochl J, Benton A, et al. A mindfulness-based intervention to increase resilience to stress in university students (the Mindful Student Study): a pragmatic randomised controlled trial. *Lancet Public Heal*. 2018;3:e72-81.
21. Chiodelli R, Mello LTN, Jesus SN, Andretta I. Effects of a brief mindfulness-based intervention on emotional regulation and levels of mindfulness in senior students. *Psicol Reflex e Crit*. 2018;31.
22. Sanada K, Montero-Marin J, Alda Diez M, Salas-Valero M, Pérez-Yus MC, Morillo H, Demarzo MM, García-Toro M, García-Campayo J. Effects of mindfulness-based interventions on salivary cortisol in healthy adults: a meta-analytical review. *Frontiers in physiology*. 2016 Oct 19;7:471.
23. Stevens BS, Royal KD, Ferris K, Taylor A, Snyder AM. Effect of a mindfulness exercise on stress in veterinary students performing surgery. *Veterinary Surgery*. 2019 Apr;48(3):360-6.
24. Tang YY, Tang R, Jiang C, Posner MI. Short-term meditation intervention improves self-regulation and academic performance. *J Child Adolesc Behav*. 2014;02.

25. Parsons EM, Dreyer-Oren SE, Magee JC, Clerkin EM. Evaluating the indirect effects of trait mindfulness facets on state tripartite components through state rumination and state experiential avoidance. *J Nerv Ment Dis.* 2019;207:1
26. Heckenberg RA, Eddy P, Kent S, Wright BJ. Do workplace-based mindfulness meditation programs improve physiological indices of stress? A systematic review and meta-analysis. *Journal of psychosomatic research.* 2018 Nov 1;114:62-71.
27. Bottaccioli AG, Bottaccioli F, Carosella A, Cofini V, Muzi P, Bologna M. Psychoneuroendocrinology-based meditation (PNEIMED) training reduces salivary cortisol under basal and stressful conditions in healthy university students: Results of a randomized controlled study. *Explore.* 2020 May 1;16(3):189-98.
28. Brown KW, Ryan RM. The benefits of being present: mindfulness and its role in psychological well-being. *J Pers Soc Psychol.* 2003;84(4):822.
29. Rayan A, Ahmad M. The psychometric properties of the mindful attention awareness scale among Arab parents of children with autism spectrum disorder. *Archives of psychiatric nursing.* 2018 Jun 1;32(3):444-8.
30. Cohen S., Kamarck T., Mermelstein R. A global measure of perceived stress. *J. Health Soc. Behav.* 1983;24:385–396.
31. Almadi T, Cathers I, Mansour AM, Chow CM. An Arabic version of the Perceived Stress Scale: Translation and validation study. *International journal of nursing studies.* 2012 Jan 1;49(1):84-9.
- 32 Levine A, Zagoory-Sharon O, Feldman R, Lewis JG, Weller A. Measuring cortisol in human psychobiological studies. *Physiology & behavior.* 2007 Jan 30;90(1):43-53.
33. Fetzer SJ. Reducing venipuncture and intravenous insertion pain with eutectic mixture of local anesthetic: a meta-analysis. *Nursing research.* 2002 Mar 1;51(2):119-24.



34. Smith JC. Relaxation, meditation, & mindfulness: A mental health practitioner's guide to new and traditional approaches. Springer Publishing Company; 2005 Mar 4.
35. Rausch SM, Gramling SE, Auerbach SM. Effects of a single session of large-group meditation and progressive muscle relaxation training on stress reduction, reactivity, and recovery. *International Journal of Stress Management*. 2006 Aug;13(3):273.
36. Ghoncheh S, Smith JC. Progressive muscle relaxation, yoga stretching, and ABC relaxation theory. *Journal of clinical psychology*. 2004 Jan;60(1):131-6.
37. Matsumoto M, Smith JC. Progressive muscle relaxation, breathing exercises, and ABC relaxation theory. *Journal of clinical psychology*. 2001 Dec;57(12):1551-7.