Brain, Behavior, & Immunity - Health 26 (2022) 100531

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Online, low-volume meditation does not alter immune-related biomarkers

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A B S T R A C T

Objectives: Prior studies of mindfulness meditation have demonstrated anti-inflammatory and immunoregulatory effects but whether meditation courses delivered online can exert similar effects is poorly understood. Barriers to large scale implementation of traditional mindfulness meditation programs has created an increased interest in the effect of less time- and resource-intensive online meditation courses. The purpose of this study was to determine whether a 6-week online mindfulness program with low time demands on nurses would lead to changes in gene expression, cytokine profiles, telomerase activity, and cortisol profiles.

Methods: This was a randomized, parallel pilot study comparing an online mindfulness-based stress management program to an active control group from December 2018 to May 2019. Healthy nurses with above average levels of perceived stress were randomized to receive a 6-week online mindfulness-based stress management program including ≥5 min daily meditation practice or listen to relaxing music for ≥5 min daily as the control arm. Blood samples were collected at baseline and after 6 weeks, and various self-reported measures of stress, physical and emotional health were collected at baseline, after 6 weeks, and after 12 weeks. Whole transcriptome mRNA sequencing of whole blood at baseline and after 6 weeks was performed along with measurement of plasma IL-6, IL-8, IL-10, TNF-α, and IFN-γ. Peripheral blood mononuclear cells were isolated, and telomerase activity was measured. Diurnal salivary cortisol profiles were assessed at baseline and after 6 weeks. The primary outcome was change over time in a pre-determined set of 53 genes representative of the immune-related changes seen with stress, which was analyzed using a mixed linear model. Secondary outcomes included all other self-reported measures and biomarkers mentioned above.

Results: A total of 61 nurses were randomized, with 52 having sufficient data to include in the final analysis. After 6 weeks, nurses in the control group reported significant reductions in stress as measured by the Perceived Stress Scale while those in the mindfulness group did not. However, after 12 weeks, the mindfulness group also showed a significant reduction in stress. When compared to the control group, no significant changes in RNA gene expression or any other biomarkers were observed in the nurses who participated in the mindfulness program.

Conclusions: Our study found that this brief online mindfulness-based intervention was effective in reducing stress in nurses, albeit with a delayed effect compared to listening to relaxing music. Regarding immunoregulatory effects, there were no significant differences between treatment and control groups in transcriptomic or other tested biomarkers of immune function. This study provides evidence for a floor effect of mindfulness on transcriptional and circulating biomarkers of immune function.

1. Introduction

Mindfulness-based interventions have repeatedly demonstrated both mental and physical health benefits in a wide variety of populations and using a variety of specific techniques (Gotink et al., 2015; Crowe et al., 2016; Grossman et al., 2004; Pascoe et al., 2017). A growing body of evidence suggests that these observed benefits are rooted in reproducible neural and molecular signatures. In brain imaging studies meditation practice is associated with changes in the morphology and activation patterns in areas of the brain responsible for attention, automatic thoughts, self-referential thinking, and emotional regulation (Marchand, 2014; Fox et al., 2014; Muehsam et al., 2017). In studies evaluating physiologic and blood biomarkers, meditation reduces heart rate, blood pressure, cortisol, CRP, TNF-α, and IL-6 and exhibits other positive immunologic effects (Pascoe et al., 2017; Meyer et al., 2019; Ng et al., 2020; Creswell et al., 2009, 2016; Black and Slavich, 2016). Furthermore, recent studies have shown a consistent pattern of alterations in gene expression in response to mindfulness-based

Abbreviations: TNF-α, tumor necrosis factor alpha; IFN-γ, interferon gamma; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; NF-κB, nuclear factor kappa B; IRF-1, interferon regulatory factor 1; CRP, C-reactive protein; CTRA, conserved ranscriptional response to adversity; PSS, perceived stress scale; PROMIS, patient-reported outcomes measurement information system.

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https://doi.org/10.1016/j.bbih.2022.100531
Received 26 May 2022; Received in revised form 8 September 2022; Accepted 1 October 2022
Available online 3 October 2022

interventions, mostly in immune-related and inflammatory pathways. In particular, mindfulness meditation appears to reduce expression of pro-inflammatory genes and NF-κB while upregulating interferon and anti-viral response genes (Muehsam et al., 2017; Buric et al., 2017). This is in direct contrast to the gene expression signature associated with chronic stress and suggests that mindfulness meditation may directly counter some of the toxic effects of chronic stress on a molecular level (Cole, 2013, 2014; Irwin and Cole, 2011).

Previous reports describing significant changes in gene expression analysis as primary outcome have largely been derived from studies using either multi-day residential retreats or time-intensive in-person interventions. Given the increasing popularity of digital mindfulness applications and programs as well as their obvious benefits in terms of accessibility, it is important that these interventions be as rigorously studied as other modalities. Several recent meta-analyses have demonstrated the potential for online mindfulness programs to reduce stress, improve mental health and, possibly, reduce self-reported physical complaints (Jayewardene et al., 2017; Toivonen et al., 2017; Spijkerman et al., 2016). Unexplored, however, is the question of what the lower limits of the mindfulness dose-response curve are in terms of its capacity to modulate immune function. No study to date has assessed the effects of an online mindfulness program on gene expression and other immune-related biomarkers. This study was designed to explore the question of whether lower frequency, online mindfulness instruction and practice over a short period of time would impact perceived stress and/or immune-related biomarkers. We chose to specifically recruits nurses for this study given the high rates of burnout and mental health disorders among this group, especially exacerbated by the recent pandemic (McHugh et al., 2011; Letvak et al., 2012; Al Maqbali et al., 2021).

2. Methods

2.1. Study design and participants

This was a single-center, single-blind, randomized, parallel pilot study comparing a 6-week online mindfulness-based stress management program to an active control that involved listening to relaxing music for at least 5 min daily (See Fig. 1). Participants were enrolled and randomized in a 1:1 ratio on a rolling basis using a random number generator. The study was approved by the Cleveland Clinic Institutional Review Board and registered in clinicaltrials.gov. Study participants were recruited from among male and female nurses and nursing assistants at the Cleveland Clinic main campus in Cleveland, Ohio from December 2018 to May 2019 through self-referral in response to posted flyers and department-wide informational emails. To be eligible to

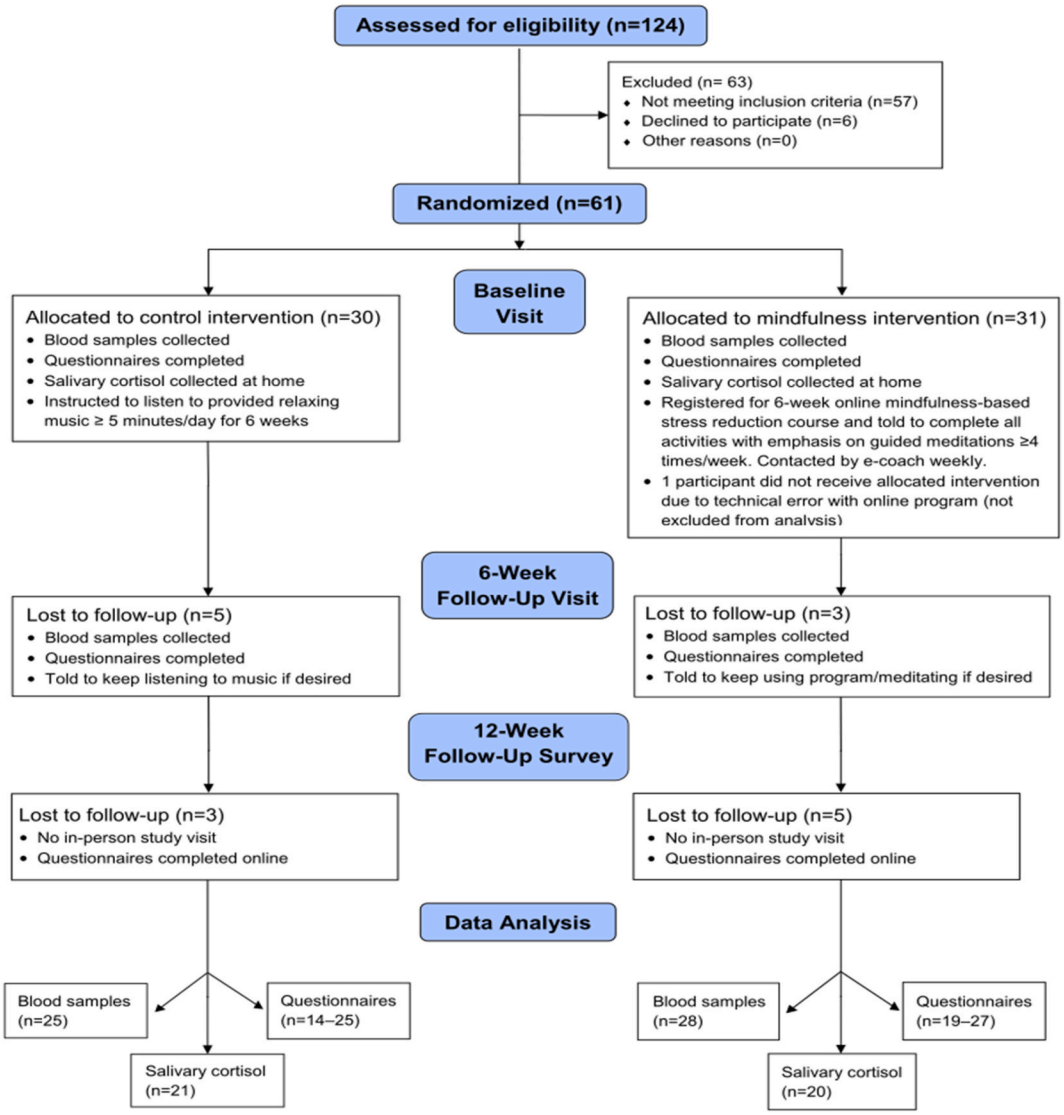


Fig. 1. Study design flow diagram.

participate they had to speak English, be able to provide informed consent, and score above average (≥7) on an abbreviated 4-question version of the Perceived Stress Scale. Participants were excluded from enrollment if they smoked, were pregnant, had any major chronic medical condition, currently used antidepressants or any other psychiatric medication, currently used hormone replacement therapy or immunosuppressive medication, or engaged in regular meditation practice either at the time of enrollment or previously.

2.2. Mindfulness and control interventions

Stress Free Now for Healers is an online stress management program developed by the Cleveland Clinic designed to reduce stress by fostering the development of mindfulness. The program includes weekly introductions of concepts, guided meditations (5–20 min each) available on the website or via the Stress Free Now for Healers iPhone app, daily articles and tips for how to manage stress or incorporate mindfulness in daily activities, and a place to record your progress. This program was modeled on a very similar online program not specifically designed for health care workers, which has been shown in a previous study to reduce self-reported stress levels over an 8-week time frame but remains unstudied in terms of biomarker analysis (Schneiderman et al., 2005). Each participant was instructed to complete as many of the daily readings, tips, and activities as possible with a particular emphasis on the meditation recordings. They were counseled that this was the key aspect of the program and that they should listen to at least one of these recordings in a quiet place without distractions every day for the next 6 weeks. The minimum frequency necessary to reap the benefits of the program was more than four times per week based on prior studies. Additionally, each participant in the intervention group was assigned an e-coach that emailed the participant at least weekly with advice and motivation and who responded to the participant's questions and concerns. At the second in-person study visit (week 6), participants in this group were told they continue to engage with the online program and meditate if they desired but were not required to do so. E-coaching stopped after week 6.

The control group was given a link to a website where they could download 27 digital recordings of relaxing music as well as flash drive containing the same recordings and were instructed to quietly listen while doing nothing else to one or more of the recordings for at least 5 min every day for 6 weeks. At the second in-person study visit (week 6), participants in this group were told they could continue to listen to the relaxing music if they desired but were not specifically instructed to do so.

All participants, whether assigned to the intervention or control group, received free life-time access to the Stress Free Now for Healers program at the conclusion of the study.

2.3. Monitoring adherence

Participants in both groups were emailed weekly with a link to complete a 1-min online questionnaire regarding either their level of participation in Stress Free Now for Healers for Healers (intervention group) or the frequency and time with which they listened to the relaxing music (control group). Additionally, website login activity for each participant enrolled in Stress Free Now for Healers for Healers was monitored and participants in the intervention group could record the number of minutes spent doing relaxation practices on the website.

2.4. Sample size determination

Estimates sample size needed was calculated for the primary outcome of change over time in gene expression between the treatment and control groups. Assuming the log of the gene intensity for each gene follows a normal distribution with (1) a mean depending on the gene, time (pre, post) and treatment (treated, control) and (2) variance equal to the coefficient of variance (cv = sd/mean) of the raw gene intensity value depending on the gene but constant across time and treatment, and assuming 15,000 genes are tested, we calculated the minimum detectable fold change between treated and control groups of 10 subjects per group. For these calculations, we used a desired power of at least 80% and an alpha (type-I) error rate per gene of 0.002215, which is equivalent to having a false discovery rate of 0.05 when 5% of the 15,000 tested genes are truly different between the treated and control groups. Assuming the correlation between pre and post gene intensity for each participant is 0.8, and the average coefficient of variation of gene intensity across all genes is 0.3, we determined that we had power to detect a minimum fold change of 1.27 between treated and control groups if we enrolled 10 subjects in each group.

Previous studies of mindfulness-based stress reduction and gene expression have successfully demonstrated >20–25% changes in more than 60 genes with around 20 subjects in each group (Chida et al., 2008; Leserman et al., 2002). Based on this data and our calculations, we planned to enroll 30 subjects in each group with sufficient power to detect a 20% minimum change in transcript level while accounting for a 10–25% dropout rate. A total of 61 subjects were ultimately enrolled with 9 of these not returning for their follow-up study visit, which is a dropout rate of about 15%.

2.5. Data and sample collection

2.5.1. Study visits

All participants were scheduled for two in-person study visits 6–9 weeks apart, each between the hours of 6 and 10 a.m. after opportunity for a full night of sleep. At each of the 2 study visits the participants had their vitals measured (height, weight, heart rate, blood pressure, and temperature) followed by a fasting blood draw. They were then administered a series of questionnaires on the computer: Global physical activity questionnaire (GPAQ), PrimeScreen (a dietary screening tool), Sleep Habits Questionnaire (4 items from Pittsburgh Sleep Quality Index regarding average sleep quantity over the past 2 weeks + Insomnia Severity Index), Perceived Stress Scale (PSS), Connor-Davidson Resilience Scale, Maslach Burnout Inventory – Human Services Survey, RAND 36 - Emotional Well-Being subscale, PROMIS Global Health (SF), PROMIS Anxiety (Computer adaptive test), PROMIS Depression (Computer adaptive test), and PROMIS Fatigue (Computer adaptive test).

2.5.2. Salivary cortisol/DHEA test

At both Study Visit 1 and 2 participants were provided with the Adrenocortex Stress Profile with Cortisol Awakening Response kit from Genova Diagnostics and told to collect 6 saliva samples at home over the course of a single day according to the provided instructions within one week of their study visit. They then mailed these samples directly to Genova Diagnostics for analysis using a prepaid envelope.

2.5.3. 12-Week follow up

12 weeks following the initial study visit participants were emailed a link to complete some of the same questionnaires they completed at the two prior study visits.

2.5.4. Laboratory procedures

All blood samples were kept at room temperature, except for the EDTA tube, which was kept on ice and immediately taken for processing, which included freezing PAXgene RNA tubes, aliquoting and freezing plasma separated from EDTA tubes, and isolation and freezing of mononuclear cells in CPT tubes. An aliquot of these cells was also lysed and frozen for the telomerase assay.

After collecting all blood samples, stabilized RNA was extracted from the PAXgene tubes (performed by CCF Genomics Biorepository) and sequenced using Next Generation Sequencing (NGS) technology (performed by CCF Genomics Core). Concentrations of IL-6, IL-8, IL-10, TNF-α, and IFN-γ in the plasma samples were measured using a multiplex

immunoassay run in duplicate with all appropriate controls and standards through the University of Maryland Cytokine Core. Telomerase activity was measured in PBMCs previously prepared with CHAPS lysis buffer using a Telomerase Repeat Amplification Protocol (TRAP) assay, which was performed by UCSF's Telomere Core.

2.6. Statistical methods

2.6.1. Demographics and subject characteristics

Categorical variables were summarized using counts and frequencies, and were compared using Fisher's exact tests. Continuous variables were summarized using median and inter-quartile range (IQR), and were compared using Wilcoxon Rank Sum tests.

2.6.2. Self-reported outcomes, cytokine levels, and telomerase activity

For the Perceived Stress Scale, Maslach Burnout Inventory, and Connor-Davidson Resilience Scale, scores at each visit and the differences of scores between the two visits (Week 6 – Week 0) as well as between Week 12 and each visit (Week 12 – Week 6, Week 12 – Week 0) were summarized using medians and IQR and were compared using Wilcoxon Rank Sum tests.

For the PROMIS Global Health, Depression, Anxiety, and Fatigue Scales, scores at each visit and the differences of scores between the two visits (Week 6 – Week 0), as well as between Week 12 and each visit (Week 12 – Week 6, Week 12 – Week 0), were summarized using means and standard deviations and compared using t-tests.

For cytokine and telomerase activity data, values at each visit and the difference between the two visits (Week 6 – Week 0) were summarized using medians and IQR and were compared using Wilcoxon Rank Sum tests.

2.6.3. Diurnal cortisol patterns

For the cortisol and DHEA data, all measures were summarized using medians and IQR at baseline and follow-up, and the difference between these measures for each subject were calculated and then averaged across the intervention and control groups. These differences were then compared using Wilcoxon Rank Sum tests. See supplementary data for details about the specific measures analyzed.

2.6.4. CTRA gene expression

While changes in the expression of individual genes in response to stress is highly variable, recent studies using genome-wide expression methods have been able to detect a reliable transcriptional signature in circulating immune cells that is consistent across studies. Mostly through the work of Steve Cole and colleagues at UCLA, this signature has been extensively studied and defined as the conserved transcriptional response to adversity, as it appears to be conserved across different types of stressors and even across species. It is characterized by an upregulation of pro-inflammatory genes and pathways, a down-regulation of interferon, antibody, and antiviral-related genes and a down-regulation of glucocorticoid signaling.

After preprocessing and assessment of read quality, RNA sequences were aligned to GRCh38 using RNA STAR version 2.7.9a using Gencode version 38 gene annotation. Raw count data was log transformed and normalized in DESeq2. The data was then analyzed using a mixed linear effect model (using package lme4 version 1.1–27.1) to determine whether there was an interaction between the subject group (intervention vs. control) and change in expression of the CTRA gene set at the 6-week compared to baseline visit, treating the 53 indicator transcripts as a single variable, with the downregulated components of the CTRA (30 interferon-related and 3 antibody synthesis genes) being sign-inverted. Associations were adjusted for potential confounding factors including age, sex, race, and BMI. See supplementary data for a detailed description of methods and R output.

3. Results

3.1. Demographics

A total of 61 nurses were enrolled in the study with 30 randomized to the control group and 31 randomized to the intervention group. Of these, there were 25 in the control group and 27 in the intervention group that completed the study. The majority of these nurses were white and female with the majority having obtained at least bachelor's degree. The median age was 33, and the median BMI was 26. See supplementary data for additional characteristics of the sample population. There were no major differences with regards to any of these characteristics between the groups.

3.2. Questionnaires

The only statistically significant changes over the study period among all the administered questionnaires was in the PSS. The initial decrease in the PSS from Week 0 to Week 6 was larger for the control group ($p < 0.001$). However, from Week 6 to Week 12 the intervention group showed a large decrease, while the scores in the control group remained relatively stable, leading to a marginally significant ($p = 0.04$) larger total decrease in perceived stress from Week 0 to Week 12 in the intervention group. No significant differences were observed between groups at Week 0, Week 6, or Week 12 nor were any of the changes over time significant for either group for any of the other self-reported

Table 1
PROMIS scores at baseline, week 6 and week 12.

		n	Control	n	Mindfulness	p-value
PROMIS Global Health	Baseline	30	34.0 ± 3.8	31	33.0 ± 3.3	0.27[a]
	Week 6	25	35.4 ± 3.2	27	34.9 ± 3.9	0.61[a]
	Week 12	22	35.7 ± 3.0	23	35.3 ± 3.2	0.68[a]
	Week 6 – Baseline	25	0.88 ± 1.8	27	2.0 ± 3.5	0.17[b]
	Week 12 – Week 6	22	0.68 ± 2.5	23	0.48 ± 2.8	0.80[a]
PROMIS Anxiety	Baseline	30	59.7 ± 5.3	31	59.8 ± 5.1	0.94[a]
	Week 6	25	52.7 ± 6.9	27	55.8 ± 6.9	0.11[a]
	Week 12	20	52.4 ± 6.9	21	55.0 ± 7.2	0.25[a]
	Week 6 – Baseline	25	−6.2 ± 5.5	27	−3.8 ± 7.1	0.19[a]
	Week 12 – Week 6	20	−0.47 ± 7.7	21	0.92 ± 6.7	0.54[a]
PROMIS Depression	Baseline	30	55.0 ± 4.8	31	54.0 ± 6.0	0.49[a]
	Week 6	25	49.5 ± 7.7	27	51.4 ± 4.9	0.29[b]
	Week 12	18	51.4 ± 6.5	20	50.4 ± 7.1	0.67[a]
	Week 6 – Baseline	25	−4.9 ± 5.7	27	−2.2 ± 6.4	0.11[a]
	Week 12 – Week 6	18	1.3 ± 7.1	20	0.18 ± 7.4	0.64[a]
PROMS Fatigue	Baseline	30	54.5 ± 7.1	31	57.8 ± 8.9	0.12[a]
	Week 6	25	49.9 ± 9.1	27	53.6 ± 8.2	0.13[a]
	Week 12	16	51.0 ± 8.4	20	51.8 ± 8.3	0.78[a]
	Week 6 – Baseline	25	−4.2 ± 7.0	27	−3.5 ± 7.0	0.72[a]
	Week 12 – Week 6	16	0.59 ± 5.9	20	−0.12 ± 5.5	0.72[a]

Statistics presented as Mean ± SD.p-values: a = *t*-test, b = Satterthwaite *t*-test.

outcomes (See Tables 1-2, Fig. 2).

3.3. CTRA gene expression

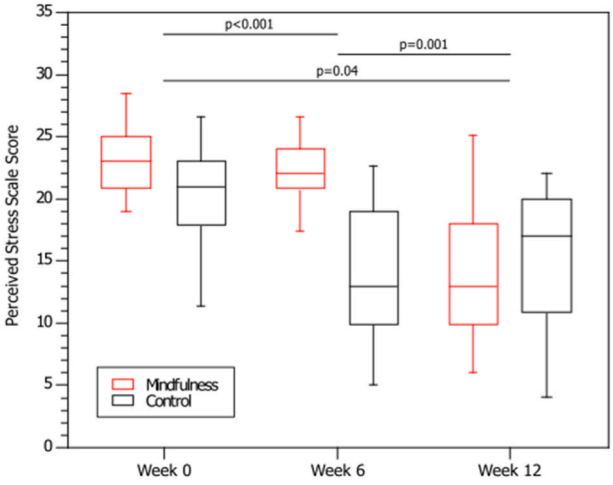
Mixed linear analysis of the CTRA gene set showed no significant interaction between time point, baseline vs 6 weeks, and group, intervention vs control (β = 0.067 [0.0092, 96.0], P = 0.89). Secondary genome-wide transcriptomic analysis revealed no association between group, PSS, or average weekly time spent meditating and changes in gene expression.

3.4. Cytokines, telomerase activity, and cortisol

At both the first and second study visits (Week 0 and Week 6), all participants had blood drawn for measurement of plasma concentrations of IL-6, IL-8, IL-10, TNF-α, IFN-γ, and CRP and telomerase activity within isolated PBMCs. Among all these measures, no statistically significant differences were observed between the intervention and control groups over the studied time period (See Fig. 3). Salivary cortisol levels over the course of one day was also measured in all participants shortly after the first and second study visits. Analysis of this data showed no difference between the intervention and control groups in terms of change in cortisol awakening response, cortisol slope, cortisol AUC, or dhea: cortisol ratio (See Table 3).

3.5. Associations between subject compliance and outcomes

To better understand the observed results and determine whether the level of participant engagement could account for some of these observations, self-reported measures of subject compliance were summarized, and linear regression between the change in PSS scores at each time point and compliance (measured in average weekly minutes spent meditating or listening to music) was performed with compliance*group as an interaction term. Furthermore, a subset analysis of the CRP data was performed on just those subjects with an average participation of four or more times per week. Measures of compliance were, overall, similar between groups with the median practice frequency being 4 and 5 times/week and the median time spent being 30 and 35 min/week in the mindfulness and control groups respectively. The one exception was in regards to the percentage of subjects averaging four or more times per week of practice, with only 46% achieving this in the mindfulness group versus 76% in the control group.



Perceived stress scores at week 0, 6, and 12. Boxes represent IQR (0.25-0.75) with whiskers representing 0.5-0.95 range. Lines within boxes represent median values. Outliers not shown for better data visibility.

Fig. 2. Change in perceived stress scale.

Multivariate regression of change in PSS, group, and compliance showed no relationship between compliance and change in PSS (β = 0.012, P = 0.70), nor was there a significant interaction between compliance and group (β = −0.088, P = 0.21).

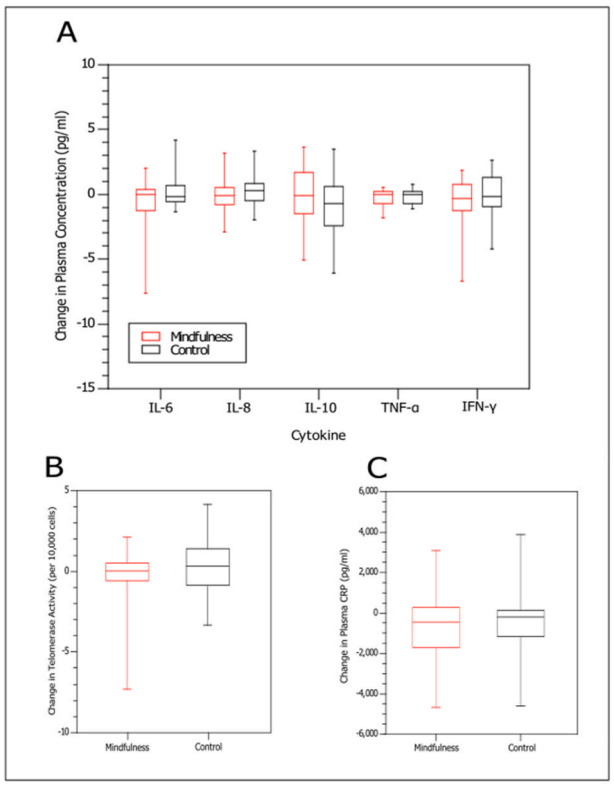
3.6. Associations between self-reported improvements in stress and outcomes

Finally, to determine whether subjects reported stress levels were related to changes in their CRP or IL-6 levels, linear regression between the change in PSS between Week 0 and Week 6 and the respective

Table 2
Perceived stress scale, maslach burnout inventory, and connor-davidson resilience scale at baseline, week 6 and week 12.

		n	Control	n	Mindfulness	p-value[c]
Perceived Stress Scale	Baseline	30	21.0 [18.0, 23.0]	31	23.0 [21.0, 25.0]	**0.010**
	Week 6	25	13.0 [10.0, 19.0]	25	22.0 [21.0, 24.0]	< **0.001**
	Week 12	15	17.0 [8.0, 20.0]	19	13.0 [9.0, 18.0]	0.57
	Week 6 – Baseline	25	−6.0 [-9.0, −3.0]	25	0.00 [-2.0, 1.00]	< **0.001**
	Week 12 – Week 6	15	−1.00 [-3.0, 6.0]	19	−8.0 [-14.0, −3.0]	**0.001**
	Week 12 – Baseline	15	−4.0 [-7.0, −2.0]	19	−9.0 [-14.0, −7.0]	**0.040**
Maslach Burnout Inventory-Emotional Exhaustion	Baseline	30	42.5 [37.0, 49.0]	31	40.0 [37.0, 49.0]	0.90
	Week 6	25	47.0 [42.0, 50.0]	25	43.0 [38.0, 49.0]	0.25
	Week 12	14	47.5 [45.0, 50.0]	19	47.0 [41.0, 51.0]	0.49
	Week 6 – Baseline	25	2.0 [-3.0, 4.0]	25	0.00 [-4.0, 5.0]	0.51
	Week 12 – Week 6	14	−0.50 [-3.0, 2.0]	19	2.0 [-2.0, 8.0]	0.38
Maslach Burnout Inventory-Depersonalization	Baseline	30	64.5 [57.0, 76.0]	31	63.0 [56.0, 67.0]	0.56
	Week 6	25	60.0 [52.0, 75.0]	25	60.0 [54.0, 65.0]	0.52
	Week 12	14	67.0 [54.0, 71.0]	19	58.0 [55.0, 68.0]	0.48
	Week 6 – Baseline	25	−4.0 [-11.0, 1.00]	25	−3.0 [-6.0, 1.00]	0.58
	Week 12 – Week 6	14	0.00 [-9.0, 4.0]	19	1.00 [-8.0, 7.0]	0.86
Maslach Burnout Inventory-Personal Accomplishment	Baseline	30	33.5 [24.0, 55.0]	31	36.0 [22.0, 49.0]	0.82
	Week 6	24	30.5 [18.5, 44.0]	25	27.0 [21.0, 37.0]	0.53
	Week 12	14	30.5 [9.0, 55.0]	19	25.0 [15.0, 46.0]	0.50
	Week 6 – Baseline	24	−5.5 [-14.0, 2.0]	25	−6.0 [-12.0, 3.0]	0.67
	Week 12 – Week 6	13	−5.0 [-8.0, 3.0]	19	0.00 [-8.0, 5.0]	0.98
Connor-Davidson Resilience Scale	Baseline	30	37.0 [34.0, 40.0]	31	37.0 [33.0, 41.0]	0.87
	Week 6	25	40.0 [38.0, 42.0]	25	38.0 [36.0, 44.0]	0.41
	Week 12	14	38.5 [35.0, 45.0]	19	42.0 [37.0, 47.0]	0.29
	Week 6 – Baseline	25	2.0 [0.00, 4.0]	25	1.00 [-2.0, 6.0]	0.52
	Week 12 – Week 6	14	0.00 [-1.00, 3.0]	19	2.0 [0.00, 7.0]	0.11

Statistics presented as Median [P25, P75].p-values: c = Wilcoxon Rank Sum test.



Change (week 6 – week 0) in a) plasma concentrations of IL-6, IL-8, IL-10, TNF-α, and IFN-γ, b) peripheral blood mononuclear cell telomerase activity, and c) plasma concentrations of CRP. Boxes represent IQR (0.25-0.75) with whiskers representing 0.5-0.95 range. Lines within boxes represent median values. Outliers not shown due to size constraints and for better data visibility.

Fig. 3. Change in circulating levels of cytokines, CRP and PBMC telomerase activity.

Table 3
Change in salivary cortisol measures.

	n	Control	n	Mindfulness	p-value
Change in Area Under the Curve (Visit 2 – Visit 1)	21	−0.23 [-1.2, 0.25]	20	0.51 [-0.41, 1.4]	0.11
Change in Slope (Visit 2 – Visit 1)	26	0.01 ± 0.02	23	−0.01 ± 0.02	0.12[a]
Absolute Change in 30 Minutes Post-Awakening Cortisol (Visit 2 – Visit 1)	20	−0.08 [-0.24, 0.06]	18	0.06 [-0.16, 0.27]	0.076[c]
Percent Change in 30 Minutes Post-Awakening Cortisol (Visit 2 – Visit 1)	20	−0.23 [-0.99, 0.40]	18	0.09 [-0.70, 0.55]	0.15[c]
Change in DHEA:Cortisol Ratio (Visit 2 – Visit 1)	21	46.0 [-169.0, 323.0]	20	−23.5 [-214.5, 91.0]	0.30[c]

Statistics presented as Median [P25, P75] except for row "Change in Slope," which is presented at mean ± SE.p-values: a = *t*-test, c = Wilcoxon Rank Sum test.

change in CRP and IL-6 was performed across all subjects from both groups. No significant associations were observed between changes in PSS and changes in CRP ($\beta = 3.3 \times 10^{-5}$, P = 0.91) or IL-6 ($\beta = -0.21$, P = 0.40).

4. Discussion

In this small pilot study, we set out to determine whether an online, 6-week mindfulness-based stress management course would result in a reduction in stress and the genomic and immunologic biomarkers of chronic stress in a group of healthy, but stressed, nurses. To our knowledge, this is the first study to investigate the effects of an online mindfulness program on immune-related biomarkers and gene expression. Contrary to our hypothesis, the blood of those who completed the course showed no change in RNA expression of the CTRA gene set as a whole, nor was there a significant change in the expression of genes regulated by NF-κB, CREB, AP-1, IRF-1 or IRF-2 as shown in previous studies of mind-body interventions (Black et al., 2013; Irwin et al., 2014, 2015; Morledge et al., 2013). Our study data are clinically important to help define a lower limit of engagement with an online mediation modality using standard meditative practices for beginners in terms of its ability to influence immune function as measured by gene analysis and other assays of inflammatory pathways.

Despite failing to provide support for our primary hypothesis, this study did demonstrate a significant reduction in levels of perceived stress in both control and intervention groups, possibly indicating a placebo or Hawthorne effect, but more likely indicating both listening to relaxing music or engaging in mindfulness practices on a regular basis can reduce stress (de Witte et al., 2022). Interestingly, while a significant reduction in stress was seen after 6 weeks in the relaxing music group, the same was not observed until the 12-week time point in the mindfulness group. Speculating as to why this might have occurred, it could be that the mindfulness program required learning and practicing new skills that lead to a delayed effect on stress compared to the more immediate effects of listening to relaxing music. It is also possible that being asked to participate in the mindfulness program was, itself, perceived as another source of stress by at least some of the participants in the mindfulness group, and that after the stress of required participation was removed at week 6, the stress-alleviating effects could be seen. Interestingly, when analyzing overall changes in perceived stress between week 0 and week 12, there was a marginally significant bigger reduction in the mindfulness group compared to the control. However, these results should be interpreted with caution due to the high dropout rate and limited available data at week 12. In fact, it is conceivable that those who responded to the emailed questionnaires at week 12 were less stressed, possibly biasing the results. It should also be pointed out that the initial median PSS in the mindfulness group was statistically higher than in the control group. However, our statistical analysis focused on the relative change over time, subtracting each subject's later scores from his/her initial scores and comparing the medians of these differences in each group, which should minimize any potential problems caused by this baseline difference in stress levels. It could still be argued, however, that these higher baseline stress levels were also more refractory to treatment, possibly making the mindfulness program appear less effective.

In terms of the capacity for mindfulness meditation practice to influence immune function as assessed by changes in gene expression and alter inflammation, the vast majority of studies demonstrating such effects have used either longer or more time-intensive in-person interventions. In a large study by Chandran et al. (2021) these included daily 45-min practice sessions plus longer, in-person weekend classes in mindfulness-based stress reduction (MBSR), week-long retreats with over 10 h of daily meditation, and concurrent dietary and lifestyle change. With the growing popularity of easily accessible online modalities for meditation practice which allow greater individual autonomy in terms of practice schedule and engagement, it is important to understand the dose-response relationship and influence of such modalities on immune function at the molecular level.

With regards to previous studies of meditation that have served to define a lower limit of practice that have demonstrated statistical change in gene expression, Black et al. (2013) were able to demonstrate a change in NF-κB and IRF1-associated gene expression with a similarly low-dose intervention that consisted of only 12 min a day of yogic meditation, but they do not provide information on the level of adherence. This makes it impossible to determine whether the level of

participation was comparable to our study. Furthermore, it is unknown whether mindfulness practices using standard basic techniques such as focused attention are equivalent to a mantra-based practice such as Yogic-meditation as employed by Black et al. (2013)

In an effort to explain the lack of any meaningful change in immune function as measured by gene expression and the immunologic and neurohormonal assays used in this study, several possibilities must be considered. First, the intensity of the practice as measured by the number of sessions and committed time may have been inadequate to effect change. Multiple previous studies of mindfulness interventions have demonstrated the importance of treatment adherence and practice time in affecting both psychological and biological outcomes (Creswell et al., 2009; Carmody and Baer, 2008; Parsons et al., 2017). Given the low level of adherence in our study, with a median weekly practice time of only 30 min in the mindfulness group and less than half the participants completing the recommended minimum of four meditation practices per week, it is possible the participants in our study did not reach the minimum amount of instruction or practice required to reduce stress or inflammation. A previous study utilizing a highly similar program incorporating the same mindfulness practices demonstrated that a weekly meditation frequency of 4.07 ± 1.59 was effective at reducing stress after 8 weeks. While the current study failed to detect a change in stress after 6 weeks, by 12 weeks there did seem to be a reduction in stress levels, indicating the intervention in this study may have been too low volume to see an effect. Interestingly, though this prior study showed a weak but significant association between the level of participation and reductions in stress levels, we did not see the same here (Morledge et al., 2013). However, our study was also underpowered to detect such associations.

Another possibility is that the duration of the intervention and the time point selected (6 weeks) resulted in sampling too soon to see a biologic effect. As noted above the documented change in perceived stress in the active arm occurred at 12 weeks but not at 6 weeks, when the patients underwent laboratory assessment. This could have limited our capacity to detect change. Comparatively, the majority of similar studies have used interventions that are at least 8 weeks long. In addition to reducing the total dose of mindfulness training, it also reduced the time for changes in biomarkers to occur. Moreover, most studies, even the ones that showed significant changes in gene expression, have not been able to demonstrate a reduction in protein-based markers of inflammation (Muehsam et al., 2017; Buric et al., 2017). Those that have shown reductions in biomarkers like CRP and IL-6 have collected follow up samples about 4–9 months from baseline (Meyer et al., 2019; Ng et al., 2020; Creswell et al., 2016). Though changes in RNA expression generally occur much faster than changes in circulating levels of protein biomarkers, more time may have been required to see a difference between the control and intervention groups, especially since changes in perceived stress levels in the mindfulness group did not change until the 12-week time point, after blood samples had been collected.

A third possibility is that our study population was not stressed enough and/or too healthy to detect a change in the selected outcomes. While we did screen for individuals experiencing higher than average levels of stress, the participants on the whole were healthier and younger than in other studies and not necessarily experiencing any particular major life stressor. This would give them a low baseline level of systemic inflammation from which it would be difficult to show significant reductions over a short time frame. It would be reasonable to repeat this study in a population with higher baseline levels of inflammation such as those with a chronic disease or mental health disorder.

One of the strengths of this study is that we used an active control group rather than a wait-list control as in many prior studies, and we showed that both listening to relaxing music and participating in an online mindfulness program reduced perceived stress of nurses, though with a delayed effect in the mindfulness group, confirming the results of a prior study of this program (Morledge et al., 2013). Although these changes did not correlate with changes in circulating biomarkers of inflammation, reducing the stress of healthcare professionals has merit in its own right given the prevalence of burn out and the greater need for resilience in the current pandemic. Additionally, this study provides evidence for a floor effect of mindfulness on transcriptional and circulating biomarkers of immune function and suggests that online programs and apps may not have all the same beneficial effects as more robust, in-person mindfulness programs and techniques. Future studies of mindfulness should focus on determining not only whether mindfulness produces psychological and biological changes but also the minimum effective dose required to produce these changes.

Funding

R.J. Fasenmyer Center for Clinical Immunology.
This study was registered at ClinicalTrials.gov: NCT03753360.

Declaration of competing interest

The online mindfulness-based stress management program studied in this trial was produced by the Cleveland Clinic, with which all the authors are affiliated. One of the authors, Dr. Calabrese, also occasionally consults with the Cleveland Clinic regarding the further development and updating of this program. However, none of the authors have any direct financial ties to the program.

Otherwise, there are no other potential conflicts of interest to disclose.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2022.100531.

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