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Psychobiological effects of a month-long Insight meditation retreat:
Implications for cell aging, neuroplasticity, and inflammatory gene expression.

By

QUINN A. CONKLIN
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Psychology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Clifford D. Saron, Chair

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Committee in Charge

2022

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In loving memory of my Boppy

cogito, ergo sum

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Cheers to the next chapter!

Abstract

In this dissertation I consider the role of psychosocial stress in mental and physical health, and investigate the role of a month-long Buddhist meditation retreat in altering the pathways between our biochemical makeup and our experience of the world.

As living beings, we possess a tremendous capacity for adaptation, and yet we are deeply conditioned by circumstance and bound by certain truths. We inhabit bodies that deteriorate, yet the rate and form of this degradation varies wildly depending on our environments and prior experiences. Stress research considers how our experiences become embedded in our bodies in ways that contribute to illness and disease, while also considering the protective factors that mitigate these outcomes. Importantly, decades of research indicate that one of the most potent modulators of health and well-being are our relationships and psychosocial environment.

Buddhist philosophy and meditation practices offer another framework for understanding our interdependence with others, and for investigating the intricate system of causes and conditions that govern our existence. Much of our conditioning and adaptation happens outside of our awareness and conscious intent, and meditation practices are often designed to bring some of this content into conscious awareness, so that we can learn to differentiate between the aspects of our lives we have some agency over and those that are inevitable.

In the Insight meditation tradition studied here, primary practices include directing one's attention to the coming and going of bodily sensations and observing thoughts as transient phenomena without grasping or over-identification. These practices are taught in combination with practices designed to support the four immeasurables: loving-kindness, compassion, empathetic joy, and equanimity. Together, these practices aim to cultivate beneficial qualities of mind and positive aspirations for oneself and others that allow practitioners to live in greater harmony with the world around them.

In the studies presented here, I examine the psychobiological effects of engaging in a month-long, silent Insight meditation retreat. Meditation retreats are an increasingly popular form of meditation training characterized by intensive or concentrated periods of meditation practice. Retreats are commonly undertaken in residential retreat settings, where practitioners are removed from the distractions of daily life. During retreat, meditators often follow a rigorous schedule of formal practice under the guidance of experienced teachers, and with the social support of fellow practitioners. This structure is designed to support practitioners in using each moment and every activity as an opportunity for continued mindfulness practice. Meditators may also adopt the practice of *noble silence*, which entails temporarily refraining from speaking, communicating, or initiating eye contact with others to facilitate quietude. Together these conditions afford a unique opportunity for participants to observe their mental experience, to cultivate particular qualities of mind, and to experience meditative insights that may have synergistic effects on their overall well-being.

Although the burgeoning field of contemplative science indicates that meditation training can positively influence physical and mental health outcomes, the biological consequences of retreats are relatively understudied. In this project, we assessed blood-based biomarkers in experienced meditators attending a month-long Insight meditation retreat (n = 28), as compared to a control group (n = 34) of experienced practitioners, similarly comprised in age and gender, who were living their everyday lives. Blood samples were collected on day two of the retreat (Time 1) and again 3 weeks later (Time 2). Control participants were also assessed across a 3-week interval, during which they maintained their regular daily routines. In the studies presented here, I report the effects of the retreat intervention on measures of 1) cell aging, as indexed by measures of telomere biology; 2) neuroplasticity, as indexed by serum levels of brain derived neurotrophic factor; and 3) inflammation, as indexed by inflammatory gene expression and transcriptional regulation.

In chapter 1, I examine changes in telomere biology, including telomere length, telomerase activity levels, and telomere-related gene expression in white blood cells. Telomeres and the enzyme telomerase interact with a variety of molecular components to regulate cell-cycle signaling cascades, and are implicated in pathways linking psychological stress to disease. I report increased telomere length in our retreat group, compared to controls who showed no group level change. Moreover, these changes in telomere length were predicted by basal personality traits such that retreat participants highest in neuroticism and lowest in agreeableness demonstrated the greatest increases in telomere length. I also report changes observed in telomere-related genes that further suggest retreat-related improvements in telomere maintenance. Although I found no group-level changes in telomerase activity, retreat participants' telomerase levels at Time 2 were inversely related to several indices of retreat engagement and prior meditation experience. Neuroticism also predicted variation in telomerase across retreat. These findings suggest that meditation training in a retreat setting may have positive effects on telomere regulation, which are moderated by individual differences in personality and meditation experience.

In chapter 2, I examine the effects of retreat on brain-derived neurotrophic factor (BDNF)—a modulatory neuroprotein implicated in learning, memory, and neuroplasticity. I then explore the possible role of neuroplasticity and BDNF on retreat-related changes in telomere length, considering the relationships between BDNF and telomere biology in the central nervous system and immune system. I found no effect of retreat on serum BDNF levels. I did, however, find a relationship between serum BDNF and moderate amounts of daily practice in control participants. I also found that BDNF at Time 1 predicted telomere lengthening during the retreat, suggesting that basal BDNF levels may play a role in retreat-related improvements observed in telomere biology.

In chapter 3, I examine the effects of retreat on the expression of 33 genes involved in inflammatory processes and cell aging. I report a pattern of gene expression in retreat participants indicative of a lower inflammatory burden—the most notable finding being consistent downregulation of the TNF-α-pathway, which was not observed in controls. These findings indicate that meditation retreat participation may influence some of the inflammatory mechanisms involved in the development of chronic diseases, and suggest that meditation retreats may have therapeutic potential, particularly for experienced practitioners.

Insights from fields as diverse as Buddhist philosophy, anthropology, biopsychology, neuroscience, and social genomics continue to demonstrate that how we perceive and experience the world shapes every aspect of our being, and, in turn, those aspects of our being affect how we influence the world around us. This study adds to these bodies of knowledge by exploring the biological changes that arise from retreats, which ideally contribute to personal growth and healing by offering opportunities for concentrated reflection, personal insight, and self-integration. As such, this dissertation grapples with how practitioners instantiate meditative learning and the health-related implications of such practice.

The design of this study was not of my making, and thus its many limitations have been a source of deep frustration over the years. However, this is now tempered by the respect I have for the daring and profound curiosity which brought this study into being. This study seeks to measure the most ephemeral of human experience with measures that are both excruciatingly crude, and some of our most sophisticated to-date. Through this study, I have learned a tremendous amount about *how* to do this work, and I hope to build on this foundation in collaboration with a wide variety of researchers, retreat centers, and practitioners.

Chapter 1

Insight Meditation and Telomere Biology:

Effects of Intensive Retreat and the Moderating Role of Personality

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ABSTRACT

Background: A growing body of evidence suggests that meditation training may have a range of salubrious effects, including improved telomere regulation. Telomeres and the enzyme telomerase interact with a variety of molecular components to regulate cell-cycle signaling cascades, and are implicated in pathways linking psychological stress to disease. **Method:** We investigated the effects of intensive meditation practice on these biomarkers by measuring changes in telomere length, telomerase activity, and telomere-related gene expression during a month-long residential Insight meditation retreat. **Results:** Multilevel analyses revealed an apparent telomere length increase in the retreat group, compared to a group of experienced meditators, similarly comprised in age and gender, who were not on retreat. Moreover, personality traits predicted changes in telomere length, such that retreat participants highest in neuroticism and lowest in agreeableness demonstrated the greatest increases in telomere length. Changes observed in telomere-related genes further suggest retreat-related improvements in telomere maintenance, including increases in *Gar1* and *HnRNPA1*, which encode proteins that bind telomerase RNA and telomeric DNA. Although no group-level changes were observed in telomerase activity, retreat participants' telomerase levels at Time 2 were inversely related to several indices of retreat engagement and prior meditation experience. Neuroticism also predicted variation in telomerase across retreat. **Conclusion:** These findings suggest that meditation training in a retreat setting may have positive effects on telomere regulation, which are moderated by individual differences in personality and meditation experience.

Keywords: meditation, telomere length, telomerase, cell aging, personality, neuroticism

INTRODUCTION

An emerging literature suggests that meditation practice and associated lifestyle interventions may promote healthy biological profiles, including alterations to stress-related physiological processes implicated in disease (Pace et al., 2009; Sudsuang, Chentanez, & Veluvan, 1991; Turan et al., 2015). Among the biomarkers potentially affected by meditation practice are telomeres (Alda et al., 2016; Hoge et al., 2013), the related enzyme telomerase (Jacobs et al., 2011; Lavretsky et al., 2013; Lengacher et al., 2014), and the expression of telomere-related genes (Bhasin et al., 2013; Duraimani et al., 2015; Epel et al., 2016).

Telomere Biology

Telomeres are nucleoprotein complexes at the ends of eukaryotic chromosomes that protect DNA from instability and degradation (Blackburn, 2000). Telomeres shorten incrementally during cell division and in response to cellular damage, making telomere length a valuable indicator of cellular aging and physiological stress (Blackburn, 1991, 2005; Harley et al., 1990). Longer telomeres are generally indicative of positive health outcomes (Blackburn, Epel, & Lin, 2015; Lin et al., 2010; Puterman & Epel, 2012), whereas shorter telomeres are associated with, and in some cases causally linked to, several degenerative and age-related diseases, such as high blood pressure and cardiovascular disease (Blackburn et al., 2015; Codd et al., 2013; Lin et al., 2010; Puterman & Epel, 2012; Shalev et al., 2013; Zhan et al., 2017).

Optimal telomere length is regulated and maintained by a complex network of molecular components collectively referred to as the telomere interactome (Robles-Espinoza, del Castillo Velasco-Herrera, Hayward, & Adams, 2015; Zheng et al., 2014). A key component of this interactome is telomerase, an enzyme that elongates telomeres by synthesizing telomeric DNA via the reverse transcriptase function of its enzymatic component, hTERT (or TERT), and its

RNA template component, hTR (also referred to as TER or TERC; Blackburn, 2005; Zheng et al., 2014). Other proteins and molecular components of the interactome facilitate correct folding of telomerase cofactors, assembly of the telomerase holoenzyme (i.e., the biochemically active compound), intracellular trafficking of telomerase, and the replication, repair, and maintenance of telomeres, among other functions.

Psychosocial Stress and Telomere Biology

Both telomere length and telomerase activity are sensitive to a variety of psychosocial and behavioral influences (Blackburn et al., 2015; Puterman & Epel, 2012; Révész, Milaneschi, Terpstra, & Penninx, 2016; Robles-Espinoza et al., 2015; Shalev et al., 2013), suggesting that links between psychological stress, health, and disease may be mediated, in part, by dynamic changes in telomere biology. For example, longer telomeres have been linked to higher levels of adaptive qualities such as conscientiousness (Edmonds, Côté, & Hampson, 2015), optimism and emotional intelligence (Schutte, Palanisamy, & McFarlane, 2016), whereas shorter telomeres have been associated with neuroticism (van Ockenburg, de Jonge, van der Harst, Ormel, & Rosmalen, 2014), anxiety (Hoen et al., 2013), and lifetime incidence of major depressive disorder (Wolkowitz et al., 2011). Additionally, perceived and biological measures of stress (Epel, 2009; Epel et al., 2004, 2010; Shalev et al., 2013; Tomiyama et al., 2012) and recent life adversity (Révész et al., 2016) are associated with shorter telomeres, and individuals who experience prolonged reactivity to stressors exhibit shorter telomeres and lower telomerase activity (Puterman & Epel, 2012). Collectively, these findings suggest that psychosocial stressors and their biological consequences contribute to telomere erosion—but that adaptive or resilient psychological profiles might offer some protection against this degradation (Schutte et al., 2016).

Meditation and Telomere Biology

Given the importance of telomere function in cellular health and longevity, interventions that mitigate telomere shortening—or promote telomere lengthening—may have profound health implications. Meditation training, which has long been theorized to improve psychological well-being is one such promising approach. Broadly defined, meditation encompasses a variety of mental training techniques drawn from a number of cultures and contemplative traditions, which—depending on technique and tradition—are intended to cultivate an array of beneficial qualities, including stability of attention, relaxation, behavioral regulation, altruistic motivations, and spiritual development (Britton & Lindahl, 2017; Dahl, Lutz, & Davidson, 2015; Lutz, Jha, Dunne, & Saron, 2015; Mehrmann & Karmacharya, 2013; Slagter, Davidson, & Lutz, 2011).

Meditation retreats are designed to facilitate concentrated training in these techniques by allowing practitioners to engage in periods of full-time intensive practice, often in a secluded environment with under the guidance of experienced teachers. Empirical evidence suggests that interventions incorporating Buddhist-derived meditation practices, in both retreat and non-retreat contexts, may reduce anxiety (Goyal et al., 2014; Platt, Whitburn, Platt-Koch, & Koch, 2016; Sahdra et al., 2011) and depression (Epel et al., 2016) and influence psychological and physiological processes implicated in stress responding (Pace et al., 2009; Rosenkranz et al., 2013; Sudsuang et al., 1991), which may in turn affect telomere regulation. Moreover, a recent review of meditation retreats suggests that there may be added benefits to practicing in the retreat format (Khoury, Sharma, Rush, & Fournier, 2015).

Ornish et al. (2008) were the first to investigate the link between meditation practice and telomerase activity, finding increased telomerase activity in prostate cancer patients following a 3-month comprehensive lifestyle intervention involving meditation. Our laboratory subsequently

corroborated this link in a non-clinical sample engaged in a 3-month residential meditation retreat (Jacobs et al., 2011). Participants of this retreat practiced *shamatha* meditation techniques (Wallace, 2006), which emphasize the development of calm, focused attention and meditative quiescence. At the end of the intervention, retreat participants exhibited higher levels of telomerase activity in peripheral blood mononuclear cells (PBMCs) as compared to a wait-list control group matched on age, gender, and meditation experience. Importantly, retreat participants' post-intervention telomerase activity levels were mediated by increases in self-reported environmental mastery (i.e., perceived control) and purpose in life (Ryff, 1989), as well as decreases in neuroticism (Goldberg, 1993)—indicating a potential link between meditation-related changes in psychological factors (e.g., Sahdra et al., 2011) and telomere regulation. Unfortunately, because telomerase activity was only measured at the end of retreat, this study was unable to rule out pre-existing group differences or to determine if telomerase activity increased over the course of the intervention (Jacobs et al., 2011). However, since these initial studies, additional research has substantiated the link between meditation and telomerase activity in both healthy adults (Epel et al., 2016; Rao et al., 2015; Tolahunase, Sagar, & Dada, 2017) and clinical populations (Daubenmier et al., 2012; Lavretsky et al., 2013; Lengacher et al., 2014; Ornish et al., 2013).

Much of the extant meditation literature has emphasized telomerase activity assessment, because changes in telomere length were thought to take months or years, which exceeds the typical duration of a meditation intervention. This view is changing, however, and researchers have begun to examine the effects of meditation practice on telomere length as well. An uncontrolled study found increased telomerase activity and a marginal increase in telomere length following a 12-week intervention involving yoga and meditation (Tolahunase et al.,

2017). Additionally, two cross-sectional studies reported meditation practitioners having longer telomeres than meditation-naive comparison groups (Alda et al., 2016; Hoge et al., 2013—in women only). Only two studies have assessed longitudinal changes in telomere length with a controlled design, and these were in cancer patients undergoing 8-week Mindfulness Based Stress Reduction (MBSR) programs (Carlson et al., 2015; Lengacher et al., 2014). Neither of these studies showed changes in telomere length in the MBSR groups; however, one found telomere length declines in controls but maintenance in the intervention groups (Carlson et al., 2015), suggesting that meditation practice may nevertheless hold protective effects. The heterogeneity of these findings suggests that the target population and the duration and intensity of a given intervention may be important explanatory variables. Furthermore, the causal relationship between meditation practice and telomere length remains to be rigorously tested in healthy participants with a longitudinal and experimentally-controlled study design.

Although the meditation interventions studied to date have varied in length, intensity, style of practice, and target population, the available evidence suggests that meditation practice promotes adaptive psychological functioning and improved telomere maintenance. In addition to telomerase activity and telomere length, meditation appears to influence the expression of several telomere-related genes (Bhasin et al., 2013; Duraimani et al., 2015; Epel et al., 2016), yet no study has directly explored the effects of meditation on a targeted array of genes involved in the broader telomere interactome. Moreover, no study has assessed changes across all three measures of cellular aging simultaneously. Because telomere length, telomerase activity, and telomere-related gene expression change and function on different time scales, they are likely to provide independent, yet complementary, information regarding intervention effects (Lin et al., 2010). For example, most intervention studies that have assessed telomere length and telomerase

activity have reported effects in one measure, but not both, suggesting that these processes may have different kinetics. Furthermore, given the aforementioned links between psychological functioning and biological processes, it is important to consider how individual differences in personality and psychological health may moderate meditation-related changes in telomere outcomes.

The Present Study

To address the aforementioned gaps in the literature, we measured changes in telomere length, telomerase activity, and an array of telomere-related genes in relation to major personality traits, anxiety, and depression during a 1-month intensive Insight meditation retreat. Within the Insight meditation tradition, practitioners engage in retreats to limit distraction and to receive guided support during periods of extended practice. While on retreat, practitioners refrain from verbal and written communication, typically eat a vegetarian diet, and are encouraged to treat all daily activities (e.g., eating, walking, simple chores) as opportunities to attend to their ongoing sensory and mental experience with open and reflexive awareness (e.g., Goldstein, 1987). From this perspective, Insight retreats can be conceived of as comprehensive well-being interventions that pair periods of formal meditation practice (e.g., sitting or walking) with a contemplative way of life or "stance toward experience" (Lutz et al., 2015). Thus, the focus of the present study is to examine the outcomes of a holistic retreat intervention that emphasizes formal meditation training.

We assessed retreat participants at the beginning of and 3 weeks into training, along with a passive control group of experienced meditators living at home. We hypothesized that both telomere length and telomerase activity would increase as a function of retreat participation. We also used a customized pathway-focused array to explore the expression of 54 genes central to

human telomerase function and telomere replication and maintenance. We expected retreat participants to demonstrate broad patterns of change in telomere-related gene expression indicative of improved telomere and telomerase function.

We also explored the degree to which individual differences in trait-like styles of thought, feeling, and action—as captured by the five-factor personality model (John & Srivastava, 1999; McCrae & Costa, 1997)—influenced practitioners' telomere-related outcomes. Because these traits represent relatively stable and enduring aspects of one's personality, we did not have strong predictions about the effects of retreat on conscientiousness, agreeableness, openness to experience, or extroversion. However, in line with the decrease observed in Jacobs et al. (2011), we expected a retreat-related reduction in neuroticism—the tendency to experience negative emotions and emotional instability (John & Srivastava, 1999). As neuroticism has been found to predict shorter telomeres (van Ockenburg et al., 2014), we further expected decreases in neuroticism to predict improved telomere regulation. We similarly expected retreat-related reductions in anxiety and depression, and that these reductions would to correlate with improved telomere biology.

METHODS

Participants

Retreat participants (n = 28; 14 female, 13 male, 1 unspecified) were recruited from a pool of candidates pre-enrolled in one of two month-long, silent residential retreats held at Spirit Rock Meditation Center (SRMC) in Woodacre, CA (February and March). To enroll in these retreats, SRMC requires individuals to have previously completed at least two silent meditation retreats of 7 days or longer. A group of comparison participants, similar in age and gender (n = 34; 23 female, 11 male), were recruited from the local SRMC community via flyers, newsletters,

and presentations at weekly community meditation gatherings. Comparison[1] participants were required to have previously attended at least two 5–10 day retreats, without having attended a retreat for at least 4 weeks prior to study participation. Applicants who self-reported major medical conditions or potentially contagious blood-borne illnesses were excluded from blood collection procedures. Given the limited pool of potential participants, individuals were not excluded for use of antidepressant or anxiolytic medication[2]. Participants gave informed consent and were paid $20 per hour for their participation. The Institutional Review Board of the University of California, Davis approved all study protocols, and the study was registered on clinicaltrials.gov (#NCT03056105).

Two retreat participants withdrew after the first assessment for reasons unrelated to the intervention, and three retreat participants did not complete questionnaires at either Time 1 or Time 2. Three control participants withdrew after the initial blood draw due to scheduling conflicts or overall time commitment, and six control participants did not complete questionnaires at one of the assessment points.

Meditation Retreat Intervention

Retreat participants were instructed in Insight meditation, a style of *vipassana* practice stemming from the Theravadan Buddhist tradition, which includes both walking and sitting variations (Goldstein & Kornfield, 2001). During sitting meditation, practitioners were asked to direct their attention to physical sensations of the body (e.g., the breath) and to observe their thoughts, desires, and intentions with gentle, reflexive awareness. During walking meditation,

[1] We use the term comparison here to denote that this was not a randomized control group; for simplicity, we refer to these participants as controls hereafter.

[2] Although a greater number of retreat participants (17%) reported use of anxiolytic or antidepressant medications than controls (5%), controlling for medication use did not change the pattern or significance of any effects, including self-reported anxiety and depression.

practitioners were instructed to bring awareness to the lifting, forward movement, and placing of their feet (Goldstein, 1987). Instruction also included the Four Immeasurables (or *Brahma Viharas* in Pali), a collection of practices used to develop benevolent feelings and motivational states towards oneself and others, including loving-kindness (*metta*), compassion (*karuna*), empathetic-joy (*mudita*), and equanimity (*upekkha*) (Salzberg, 1995; Wallace, 1999).

Retreats were taught by teams of six experienced teachers and held in "noble silence," meaning participants refrained from regular verbal and written communication, as well as eye contact, except during periodic meetings with teachers. Participants lived on-site for the duration of the retreat and were provided an ovo-lacto vegetarian diet. The daily schedule included alternating periods (30–45 mins) of sitting and walking meditation, totaling roughly 10 hours per day, along with work meditations (i.e., chores) and regular meal times (see Supplementary Appendix A for daily retreat schedule). Seated meditation practice was held in a spacious meditation hall, where approximately 80 retreat participants meditated as a group, and walking meditation took place in adjoining halls or outdoors on the SRMC grounds.

Assessments

Retreat participants were assessed at SRMC the morning following their first full day of silent meditation, and again 3 weeks later (several days before ending silence). Control participants were assessed in waves from May through February of the following year, at the Anubhuti Retreat Center (Novato, CA), a similarly peaceful setting in the same geographic region. Groups of 4–12 control participants were assessed at the beginning and end of 3-week intervals, during which they maintained their normal daily routines. Retreat participants gave blood between 5:00 and 6:00 am. Control participants completed a 40-minute meditation session prior to each blood collection, and gave blood between 9:00 and 10:00 am. Professional

phlebotomists collected blood via antecubital venipuncture, and all participants were asked to refrain from eating for 8 hours prior to the draw. Participants' weight was measured immediately before each blood collection, and their height was recorded at the second assessment. Body mass index was calculated as BMI = [Weight (lbs) * 703] / Height $(in)^2$. Following each blood collection, participants were given a packet of questionnaires, which were completed and returned to the experimenters within 36 hours.

Blood Sample Preparation

Whole blood was collected in Vacutainer Cell Preparation Tubes (Becton Dickinson, Franklin Lakes, NJ) and transported at room temperature to a field lab where PBMCs were isolated by density-gradient centrifugation (Sorvall Legend) as per manufacturer's instructions (time from collection to centrifugation was less than 30 min). Cells were washed twice with Dulbecco's phosphate buffer saline and stained with Trypan Blue to count live cells using a hemocytometer. For telomere length measurement, ~1 million PBMCs per sample were pelleted and stored at -80°C. For telomerase activity assays, aliquots of 0.5 million cells were pelleted and lysed with 1×CHAPS buffer. Extracts containing 5000 cells/µl were then prepared and stored at -80°C. Samples were subsequently delivered on dry ice to the University of California, San Francisco, where telomerase activity and telomere length were assayed in the laboratory of Dr. Elizabeth Blackburn. For gene expression, cell pellets of ~2 million cells were conserved in RNA Later (Sigma, St Louis, MO) and stored at −80°C, then shipped on dry ice to the University of Barcelona for RNA extraction in the laboratory of PK.

Telomere Length Measurement

Total genomic DNA was purified from PBMCs using QIAamp DNA Mini kits (QIAGEN, Cat# 51104) and stored at -80°C for batch telomere length measurement. The

telomere length assay methodology was identical to that of Lin et al. (2010; adapted from Cawthon, 2002, 2009), with the exception that reaction mixes contained 1.78–12.6 ng of genomic DNA per 11 µl reaction.

Telomere length is expressed as t/s, the ratio of telomeric (T) to single copy (S) gene product for a particular sample. T and S values were measured in triplicate using a real-time PCR machine with a 384-tube capacity, and then averaged before calculating the t/s ratio for a given sample from a given PCR batch or 'run'. Each sample was measured in two to five runs, resulting in two to five t/s values per sample (M = 3.7 values, SD = 1), which were then adjusted for inter-assay variability between runs using a normalizing factor derived from 8 different DNA samples of control cancer cell lines. These control samples were measured in quadruplicate in each present PCR batch to derive an averaged t/s value; this value was then divided by the average t/s value for the same DNA from 10 prior PCR batches to obtain a normalizing factor. This was done for all 8 control samples and the average of these normalizing factors was used to correct the participant DNA samples to get the final t/s ratios. The inter-assay coefficient of variation was 4.7%.

Steenstrup et al. (2013) have suggested that most, if not all, instances of observed telomere lengthening may be attributable to measurement error. For instance, a common analytic approach is to measure t/s ratios two or three times per sample and to average these values to obtain a single estimate of telomere length. However, averaging t/s ratios may obscure inter- and intra-assay variability. To better account for these sources of error, we used a multilevel modeling approach in which raw t/s values were nested within participants and assay runs.

Telomerase Activity Measurement

Telomerase activity was measured using Gel-TRAP (Telomerase Repeat Amplification Protocol) assays. Reactions were carried out according to the TRAPeze Telomerase detection kit (Millipore, Cat# S7700) and run on an 8% polyacrylamide-8M urea sequencing gel. Gels were then exposed to a phosphorimager plate overnight and scanned on a Typhoon 8600 Imager (GE Healthcare, Piscataway, NJ). 293T cancer cells were used as a positive telomerase activity control and standard. Telomerase activity was expressed as the equivalent number of 293T cells and quantified using ImageQuant 5.2 software (GE Healthcare, Piscataway, NJ). Signals from product ladders were added and normalized against the signal from an internal control band for the same lane of the gel to calculate the product/internal control value. For each assay reaction, the product/internal sample value was divided by the product/internal control value from twenty 293T cells and multiplied by 20 to obtain the final telomerase activity units, where 1 unit equals the product from one 293T cell/10,000 immune cells. The average intra-assay variability of PBMC samples was 8% (from six samples assayed in triplicate) and the inter-assay variability of PBMC samples was 6.7% (from 24 samples assayed on two different days). To minimize batch differences, Time 1 and Time 2 samples from each participant were assayed in the same batch, with batches containing approximately equal numbers retreat and control samples. Lab personnel performing assays were blind to group status.

Gene Expression

A list of 54 telomere-related candidate genes was curated based on existing literature indicating their mechanistic role in telomere maintenance via personal communication with Dr. Blackburn. Total RNA was extracted using the mirVana™ RNA Isolation Kit (Applied Biosystems) as per manufacturer's instructions. Yield, purity, and quality of RNA were

determined spectrophotometrically (NanoDrop, USA) using Bioanalyzer 2100 (Agilent Technologies) capillary electrophoresis, resulting in RNA with 260/280 nm ratios above 1.9 and RNA Integrity Numbers (RIN) higher than 7.5. Random-primed cDNA synthesis starting with 0.2 μg of RNA was performed at 37 °C using the High-Capacity cDNA Archive Kit (Applied Biosystems).

Quantitative real time (q-RT) PCR was performed using a Bio-Rad CFX384 real-time PCR system and TaqMan FAM-labeled specific probes (Applied Biosystems) listed in Table S3. Duplicates were run for each sample on a 384-well plate. A pre-amplification step (Taqman PreAmp Master Mix; Applied Biosystems, Foster, CA) was performed for genes exhibiting very low levels of expression (*Ctc1, Obfc1, Terf1, Tert* and *Xrcc2*). Q-RT PCR data were analyzed with the Bio-Rad CFX Manager using the automatic setting to determine the Ct baseline and threshold. Relative expression of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method after normalizing to TBP reference gene expression (Livak & Schmittgen, 2001).

Self-Report Measures

Lifestyle Profile II. Health responsibility (9 items), physical activity (8 items), nutrition (9 items), spiritual growth (9 items), interpersonal relations (9 items), and stress management (8 items) were assessed with the 52-item Lifestyle Profile II (Walker, Sechrist, & Pender, 1987). Participants indicated the frequency with which they engaged in listed behaviors using a 4-point Likert-type scale ranging from “Never” to “Routinely.” Subscale scores were averaged, with higher scores indicating more frequent engagement with a particular lifestyle factor.

State Trait Anxiety Inventory (STAI-T). Trait anxiety was assessed with the 10 trait items from the STAI (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). Respondents indicated how they felt generally over the past month on a 4-point Likert-type scale ranging from

"Almost Never" to "Almost Always." Responses were summed for each participant, with higher scores indicating greater trait anxiety.

Center for Epidemiologic Studies Depression Scale (CES-D). Depression symptoms were assessed with the 20-item CES-D scale (Radloff, 1977). Respondents indicated the frequency with which they experienced aspects of depression, such as poor appetite, restless sleep, and feeling lonely over the last month using a 4-point Likert-type scale ranging from "Rarely or none of the time (less than 1 day each week)" to "Most or all of the time (5–7 days each week)." Responses were summed for each participant, yielding a possible range of 0 to 60, with higher scores indicating greater depressive symptoms.

Big Five Inventory (BFI). Personality dimensions were assessed using the 44-item Big Five Inventory (Goldberg, 1993): extraversion (8 items), agreeableness (9 items) conscientiousness (9 items), neuroticism (8 items), and openness to experience (10 items). Respondents indicated the degree to which they agreed with the items listed on a 7-point Likert-type scale from "Strongly Disagree" to "Strongly Agree." Subscale scores were averaged, with higher scores indicating a greater degree of the given personality dimension.

Statistical Analyses

Multilevel mixed effects models (MLMs) were used to test changes in dependent measures over time and to examine relations between self-report and cell aging measures. Models assessing changes in telomere length, telomerase activity, telomere-related gene expression, and self-report measures included the fixed effects of group (control = 0, retreat = 1) and time (Time 1 = 0, Time 2 = 1), and the interaction between group and time. Random effects for participants were included to allow for within-person dependency across assessments. Analyses assessing telomere length also included a random effect of assay run to account for

variance common to samples run in the same batch. Because MLMs can accommodate missing data, participants who dropped from the study or were otherwise unable to complete all assessments were included in analyses.

Models were estimated with PROC MIXED in SAS Version 9.4 using restricted maximum likelihood. Significance of fixed effects was evaluated using Kenward-Roger approximated degrees of freedom reported to the nearest integer. Log-likelihood tests of change in model fit (-2ΔLL) were used to test significance of random effects. The normality of model residuals was tested using the Shapiro-Wilk test, and homogeneity of variance was assessed by comparing models assuming equal variance to those allowing for heterogeneous variance between groups and assessment points. Telomerase activity and telomere-related gene expression levels were log-transformed to meet normality assumptions.

In line with prior research (Cramer & Imaike, 2002; Müezzinler, Zaineddin, & Brenner, 2014; Müezzinler, Zaineddin, & Brenner, 2013; Robles-Espinoza et al., 2015; Savolainen, Eriksson, Kajantie, Pesonen, & Raikkonen, 2015), age, gender, and BMI were considered as covariates in statistical models of telomere length and telomerase activity. Age was centered at the grand mean (50.74 years), and gender centered to female (female = 0, male = 1). In order to differentiate the effects of baseline differences in BMI from retreat-related changes in BMI, we partialed this predictor into Time 1 assessment BMI (centered at the grand mean, M = 24.16 BMI) and change in BMI (Time 2 BMI – Time1 BMI). Non-significant covariates were removed from models before proceeding with further analyses.

Post-hoc mean comparisons of telomere length, telomerase activity, and self-report measures were adjusted using the Tukey-Kramer procedure. For analyses of telomere-related genes, pairwise comparisons of model-estimated mean differences within each group over time

(time 1 – time 2) and between groups at each assessment (retreat − control) were extracted, yielding 216 total test statistics (4 comparisons × 54 genes). The pool of resultant *p*-values was then subjected to false discovery rate (FDR) control of Type I error (Reiner, Yekutieli, & Benjamini, 2003) using the two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli (2006). The expected proportion of false discoveries (Q) was set at .05, and observed (uncorrected) *p*-values are reported for all analyses.

RESULTS

Participant demographics and lifestyle variables are reported in Table S1. Prior meditation experience variables are reported in Table S2. Groups did not differ in age, gender, BMI, or lifestyle factors at Time 1, nor in reported lifetime meditation experience, total number of retreats, or longest previously attended retreat (all *p*s > .05). Retreat participants had, however, attended significantly more days of retreat in the prior year than controls (Control $M = 10$, Retreat $M = 28$, $p = .016$). BMI decreased across assessments in retreat participants (ß = -0.632, SE = 0.13, $p < .0001$), but not in controls. Observed means and standard deviations for telomere length, telomerase activity, and self-report measures are reported in Table 1.1, with corresponding alpha reliability coefficients for each scale.

Telomere Length

To characterize sources of variability in t/s ratios, we first fit an unconditional MLM with individual t/s values at level 1 cross-nested within participants and assay runs at level 2. There were two to five t/s values for each participant at each assessment, totaling 445 observations. Inclusion of a random intercept for participants significantly improved model fit over the unconditional model [$-2\Delta LL\ (1) = 681.2$, $p < .001$], as did the inclusion of a random intercept for assay variability [$-2\Delta LL\ (1) = 73.1$, $p < .001$]. The intraclass correlation coefficient (ICC) for the

participant intercept was 0.86, suggesting that 86% of the total variance in t/s values was attributable to differences between individuals. The ICC for assay run indicated that 3% of the variance in t/s ratio was due to variability between assay runs, leaving 11% of the total variance attributable to unexplained within-person or within-assay factors. The majority of variance in t/s ratios was thus attributable to differences between, rather than within, individuals. Nevertheless, assay run explained an additional 3% of variance that would otherwise contribute residual error to the data, confirming the utility of using this MLM approach.

Next, we examined retreat-related changes in telomere length. There was no effect of group [$F(1, 59) = 2.22$, $p = .141$], but there was a significant effect of time [$F(1, 377) = 9.12$, $p = .003$], and a significant interaction between group and time [$F(1, 378) = 11.65$, $p < .001$], such that retreat participants showed an estimated increase of 0.046 units in t/s ratios across assessments [$p < .001$, 95% CI (0.019, 0.072)], compared to controls. Mean comparisons similarly affirmed a statistically significant increase of 0.043 units within the retreat group from Time 1 to Time 2 [$p < .001$, 95% CI (0.024, 0.063)], reflecting an apparent increase in telomere length of approximately 104.2 base pairs (bp)[3]. There was no change in controls (all ps > .05), and no significant difference between groups at either assessment. Finally, we examined the effects of person-level covariates on t/s ratios. Age, gender, and BMI (change and baseline) each predicted telomere length when entered separately into the model (see Table S4 for model parameters). However, BMI was not a significant predictor of t/s ratios when BMI and gender were both included as predictors; thus, the final model assessing telomere length included only age and gender as covariates (see Base Model in Table S5). Parameter estimates indicated that

[3] The formula bp = 3274+2413*(t/s) was used to convert t/s ratios to base pairs (72), which was derived from a direct comparison of qPCR t/s ratios and Southern blot analyses performed in the lab of Dr. Blackburn on a set of DNA samples from cultured human fibroblasts.

each year of age corresponded to a decrease in telomere length of -0.009 units [$p < .001$, 95% CI (-0.013, -0.006)], and that male telomere length was, on average, -0.135 units [$p = .001$, 95% CI (-0.214, -0.055)] shorter than females, consistent with other studies (Gardner et al., 2014; Robles-Espinoza et al., 2015). Importantly, the interaction between group and time remained significant after controlling for covariates in this model [$F(1, 372) = 12.89$, $p < .001$].

Telomerase Activity

Contrary to our hypotheses, there was no significant effect of time [$F(1, 57) = 2.59$, $p = .113$] or interaction between group and time [$F(1, 57) < 0.01$, $p = .975$] on telomerase activity. We did observe a significant main effect of group [$F(1, 60) = 4.76$, $p = .033$], such that the control group had higher levels of telomerase activity across assessments (Figure 1.1); however, post-hoc comparisons indicated that the difference between groups was not significant at either assessment (ps > .05). Neither age [ß = -0.002, 95% CI (-0.013, 0.008)] nor gender [ß = 0.073, 95% CI (-0.199, 0.345)] significantly predicted telomerase activity when covariates were included in the model. Similarly, neither Time 1 BMI [ß = -0.007, 95% CI (-0.042, 0.03)] nor change in BMI [ß = 0.251, 95% CI (-0.031, 0.534)] predicted telomerase activity; however, when included, these predictors eliminated the previously observed group difference.

Relations between Telomerase Activity, Telomere Length, and Retreat Experience

In line with previous studies (Lin et al., 2016, 2010), telomere length and telomerase activity were not significantly correlated at either Time 1 [$r(58) = .17$, $p = .198$] or Time 2 [$r(51) = -.19$, $p = .163$] in the overall sample. Changes in telomerase activity were also uncorrelated with changes in telomere length across assessments [$r(49) = .09$, $p = .545$]. However, baseline telomerase activity was negatively correlated with changes in telomere length across assessments, such that lower telomerase activity at Time 1 was associated with greater

increases in telomere length across groups [$r(52) = -.28$, $p = .043$). This correlation was significant in retreat participants when considered separately [$r(24) = -.41$, $p = .038$], but not in controls [$r(26) = -.12$, $p = .552$]. To further characterize this relationship, we entered telomerase activity, time, and the interaction between telomerase activity and time into a model predicting telomere length in retreat participants only, with age and gender included as covariates. This model revealed a significant interaction between telomerase activity and time, [$F(1, 167) = 10.63$, $p = .001$]. According to simple slope estimates, retreat participants at the retreat group mean [ß = 0.045, 95% CI (0.025, 0.065) or 1 SD below the retreat group mean [ß = 0.091, 95% CI (0.059, 0.123)] were estimated to have significant increases in telomere length across assessments.

We also explored relations between telomere length, telomerase activity, and retreat participants' reports of practice engagement during the retreat intervention (see Supplemental Material for a full description of practice engagement measures, and supplemental correlations with lifetime meditation experience variables). Changes in telomere length were positively correlated with retreat participants' reports of how diligently they had practiced in relation to their peers, Spearman $r_s(22) = .42$, $p = .039$, whereas telomerase activity levels at Time 2—but not changes in telomerase activity—were negatively correlated with reports of practice diligence, $r_s(22) = -.57$, $p = .003$, telomerase activity at Time 2 was also negatively correlated with retreat participants' estimates of the number of hours they had spent meditating during retreat, $r(23) = -.43$, $p = .033$.

Telomere-Related Genes

To explore molecular mechanisms underlying patterns of telomere length and telomerase activity, we investigated retreat-related changes in 54 candidate genes. Significant model-

estimated pairwise comparisons and resultant *p*-values are presented in Table 1.2; non-significant gene results are presented in Table S6. Significance was evaluated at an FDR threshold of .018 across all tests; *p*-values below this threshold are interpreted as discoveries.

Retreat participation appeared to impact group differences in gene expression across assessments: of the 54 candidate genes, only six (11%) showed group differences at Time 1 (*Cct2, Cct4, Terf1, Terf2, Upf3b, Xrn1*), whereas 24 (44%) showed group differences at Time 2 (see Table 1.2). The retreat group exhibited significant changes in 17 genes (31%) that did not differ between groups at Time 1. Of this subset, *Atrip, Cct1, Cct6, Gar1,* and *Hnrnpa1* showed increased expression levels in retreat participants but not controls, whereas *Nabp1*, *Pot1*, *Wrap53, Xrcc3,* and *Xrcc4* showed decreases in retreat participants only. Seven genes showed changes in both groups: *Cct1* and *Nabp2* increased in retreat participants but decreased in controls; *Upf1* decreased in both groups, with significantly lower expression in retreat participants at post; and *Atmin*, *Nhp2*, *Tep1*, and *Tinf2* changed in both groups, but did not differ at Time 2.

An additional 11 genes (*Atm, Atr, Atrx, Cct2, Cct7, Cct8, Dkc1, Obfc1, Upf3a, Xrcc6, Xrcc6bp1*) showed decreased expression levels in controls only. Significant group differences were observed at Time 2 for eight of these genes. Of these, only *Cct2* showed a significant group difference at Time 1. There were no significant changes in the remaining 28 genes for either group.

Self-Report Measures

We assessed changes in self-report measures across groups and assessments, and observed significant group by time interactions for trait anxiety [$F(1,51) = 5.18$, $p = .027$; Figure S1] and conscientiousness [$F(1,48) = 11.43$, $p = .001$, Figure S2] only. Post-hoc comparisons

revealed a significant decrease in trait anxiety [ß = -3.79, 95% CI (-7.24, -0.34)] and an increase in conscientiousness [ß = 0.26, 95% CI (0.05, 0.48)] in the retreat group, but no changes in controls and no significant differences between groups at either assessment (all *p*s > .05). There were also significant main effects of time for neuroticism [$F(1,49 = 6.49, p = .014$] and agreeableness [$F(1,51) = 4.07, p = .05$]. Neuroticism decreased [ß = -0.21, 95% CI (-0.38, -0.04)], while agreeableness increased [ß = 0.29, 95% CI (0.001, 0.27)], in both groups from Time 1 to Time 2 (Figure S2). No significant effects were observed for openness, extraversion, or depression (all *p*s > .05).

Relations of Self-Report Measures to Telomere Length and Telomerase Activity

Inspection of individual change scores revealed substantial variability in the magnitude of telomere length changes (Figure S3). To better characterize these individual differences, we fit a series of models assessing relations of self-reported personality factors, anxiety, and depression to telomere length and telomerase activity.

We first assessed the main effects of self-report variables in predicting telomere length overall (i.e., across assessments and groups). Each predictor was added to a separate model including fixed effects of group, time, and the group by time interaction, with age and gender as covariates (Table S5). Anxiety [ß = -0.002, 95% CI (-0.004, -0.001)] and depression [ß = -0.002, 95% CI (-0.005, -0.0002)] each significantly predicted telomere length, with higher levels relating to shorter telomere length. There was a similar, yet non-significant, effect of neuroticism [ß = -0.019, 95% CI (-0.039, 0.001)]. No effects were observed for agreeableness, openness, conscientiousness, or extraversion.

Next, we assessed whether self-report measures interacted with group and time to predict telomere length. Significant three-way interactions were found for neuroticism, agreeableness,

and openness (Table S5). To interpret these interactions, we estimated simple slopes at 1 SD below and 1 SD above the mean for each significant interaction. As shown in Figure 1.2, model estimates indicated greater telomere length increases in retreat participants 1 SD above the mean in neuroticism [ß = 0.094, 95% CI (0.052, 0.136)] or 1 SD below the mean in agreeableness [ß = 0.138, 95% CI (0.081, 0.194)]. By contrast, estimates indicated little change in telomere length for individuals 1 SD below the mean in neuroticism [ß = -0.018, 95% CI (-0.068, 0.033)] or above the mean in agreeableness [ß = -0.009, 95% CI (-0.054, 0.036)]. Model estimates also indicated telomere lengthening in retreat participants who were 1 SD above the mean in openness [ß = 0.077, 95% CI (0.035, 0.119)]—but shortening in controls [ß = -0.040, 95% CI (-0.077, -0.003)].

Parallel analyses were conducted with telomerase activity as the outcome variable. In these models, no psychological variable predicted telomerase activity as a main effect. When interaction effects were added, we observed significant two-way interactions between neuroticism and group [$F(1,69) = 7.45$, $p = .008$] and neuroticism and time [$F(1,49) = 11.01$, $p = .002$], but a non-significant three-way interaction between neuroticism, group, and time [$F(1,49) = 3.27$, $p = .077$]. Simple slopes, depicted in Figure S4, show a significant decline in telomerase activity from Time 1 to Time 2 in retreat participants 1 SD below the mean in neuroticism [ß = -0.780, 95% CI (-1.334, -0.226)]. For participants 1 SD above the mean in neuroticism, estimates indicated that retreat participants had significantly lower telomerase activity at Time 1 [ß = -0.703, 95% CI (-1.286, -0.131)], but did not significantly differ from controls at Time 2. Thus, retreat participants who entered retreat low in neuroticism seem to have decreased in telomerase activity, while those higher in neuroticism seem to have shown a subtle increase, which is logically consistent with the pattern of observed telomere results.

These analyses suggest that both agreeableness and openness moderate retreat-related changes in telomere length, whereas neuroticism appears to moderate changes in both telomere length and telomerase activity. However, because these models included self-report variables as time-varying predictors, we could not differentiate whether changes in telomere length or telomerase activity were driven by interindividual differences in baseline personality measures, or by intraindividual changes across retreat. Therefore, for each self-report index, we partialed baseline self-report values (Time 1 scores, centered at the grand mean) from changes in self-report (Time 2 – Time 1 difference scores). We then entered these new variables as predictors in models of changes in telomere length or telomerase activity (Time 2 – Time 1 difference scores) for retreat participants only. A significance criterion of .025 was used to adjust for potential inflation of Type I error that can result from using dependent baseline and change scores as simultaneous predictors.

Baseline neuroticism [$F(1, 21) = 9.80$, $p = .005$] and agreeableness [$F(1,21) = 9.51$, $p = .006$] each significantly predicted change in telomere length across retreat. Neuroticism was positively related to change in telomere length: individuals who entered retreat with higher neuroticism scores showed greater increases in telomere length across retreat (Table 1.3; Figure S5, top). This finding does not appear to be the result of a floor effect—wherein individuals higher in neuroticism had greater room for improvement due to shorter telomeres at baseline—as neuroticism and telomere length were unrelated at Time 1, $r(24)= -0.23$, $p = .263$. Baseline neuroticism was also positively related to changes in telomerase activity across retreat [$F(1, 21) = 10.47$, $p = .004$; Table 1.3], consistent with the patterns observed in Figure S4. Agreeableness, on the other hand, was negatively related to changes in telomere length, such that participants who entered retreat with lower agreeableness scores were more likely to show increased

telomere length (Table 1.3; Figure S5, bottom). Both neuroticism [ß = 0.023, 95% CI (0.008, 0.037)] and agreeableness [ß = -0.062, 95% CI (-0.094, -0.029)] remained significant predictors of telomere length when entered into the model simultaneously—despite a significant negative correlation between the two at baseline (r = -.53, $p < .0001$)—indicating that these variables independently predicted change in telomere length. Interestingly, while baseline neuroticism and agreeableness predicted changes in these cellular measures, *changes* in self-reported personality measures were unrelated to changes in telomere length or telomerase activity.

DISCUSSION

To our knowledge, this study is the first to report an apparent increase in telomere length within a 3-week intervention period, suggesting that meditation retreats may be a powerful method for improving cellular longevity. In addition to increased telomere length, we found that changes in telomere length, as well as patterns of telomerase activity, were moderated by individual differences in personality. We also observed retreat-related changes in the expression of a number of genes implicated in telomere biology, including those that promote intracellular trafficking of telomerase; correct folding of telomerase cofactors; and replication, repair, and maintenance of telomeres.

Increased Telomere Length

The mean telomere length increase observed among retreat participants in our study was equivalent to approximately 104.2 bp. A systematic review of the relationship between age and leukocyte telomere length across 129 studies revealed an average yearly telomere loss of 24.7 bp (Müezzinler et al., 2014). Consistent with this literature, telomere length in the present study was estimated to shorten by an average of 22.9 bp for each additional year of age. Thus, the apparent telomere increase observed in the retreat group was equivalent in magnitude to the decline

typically observed over about 4 years of aging. Although these findings support the view that retreat participation may boost physiological outcomes related to healthy aging, it is unknown whether this short-term increase is maintained beyond the intervention period. It is also important to note that, due to differences in assay methodology and reference standards, the conversion factors used to estimate base pairs from t/s ratios vary between labs; therefore, comparisons should be interpreted cautiously. Nevertheless, as a proof-of-concept, our findings demonstrate the malleability of telomere length, given the dramatic increase observed in just 3 weeks.

Telomerase Activity

In contrast to the results of Jacobs et al. (2011), in which retreat participants had greater levels of telomerase activity than controls at the end of the intervention, we observed slightly higher levels of telomerase activity in controls overall—though this difference was not statistically significant at Time 1 or Time 2 assessment. Epel et al. (2016) also found lower levels of telomerase activity in experienced meditators compared to meditation-naive individuals at baseline. Interestingly, we found that retreat participants' telomerase activity levels at Time 2 were inversely related to measures of retreat engagement, and to indices of prior meditation experience (reported in Supplementary Material). Practitioners who were more experienced tended to have lower telomerase levels after 3 weeks of retreat. Retreat participants also reported greater amounts of retreat practice (in days) in the prior year. One possible explanation for this pattern of lowered telomerase activity but longer telomeres is that experienced meditators may require less telomerase activity to maintain optimal telomere length, because they are able to mitigate the effects of stressful situations before incurring telomere damage. This hypothesis is supported by a recent study showing that higher allostatic load and impoverished psychosocial

resources are related to the opposite pattern of telomere biology, that is shorter telomeres and higher telomerase levels (Zalli et al., 2014). However, in the present study, both groups were drawn from a pool of experienced meditators, and thus we can only speculate regarding the small group difference in telomerase activity. Moreover, group assignment was not randomized, making it difficult to completely rule out pre-existing group differences, including possible lifestyle factors that enabled retreat participants to leave work and family commitments for a month-long retreat.

Given the heterogeneity of telomerase activity findings in the literature, it is difficult to discern a clear relationship between meditation practice and telomerase activity across studies. Many studies reporting an increase in telomerase activity have used one-tailed tests, assuming that telomerase activity will increase with meditation practice. However, the present results and those of Epel et al. (2016) suggest that telomerase activity may actually be lower, or even decline, in some meditation practitioners. It is also notable that several studies have reported significant findings in active control groups, suggesting telomerase activity may not be sensitive to meditation specifically, but to health-enhancing interventions more generally (Carlson et al., 2015; Daubenmier et al., 2012; Duraimani et al., 2015). Finally, these contradictory findings may be an artifact of the different timescales at which telomerase activity has been measured across studies. Telomerase activity is labile and can change on the order of an hour (Epel et al., 2010). Thus, it is plausible that the measurement occasions in the current study—spaced three weeks apart—masked short-term changes in telomerase activity that may have, nonetheless, contributed to observed increases in telomere length. For example, experienced meditation practitioners may have lower baseline levels of telomerase that increase within the first week of a retreat (in line with Epel et al., 2016), and then decline again once telomeres have been lengthened. This notion

is supported by the observation that telomere length increases in our data were related to lower baseline telomerase levels. Rapid changes in telomerase activity may also explain the lack of correlation between telomerase activity and telomere length at any single assessment, observed here and elsewhere (Lin et al., 2016, 2010). To better elucidate dynamic changes in telomerase activity across interventions, future studies will benefit from incorporating active control groups, two-tailed statistical tests, and additional assessment points with multiple samples collected at each assessment.

Telomere-related Gene Expression

Although we did not detect changes in telomerase activity across retreat, we did observe significant changes in the expression of several genes that encode proteins involved in the telomerase holoenzyme and its assembly, including *Gar1*, *HnRNPA1*, and *Dkc1* (Ford et al., 2002; see Wu et al., 2017 for review and illustration). Both *Gar1* and *HnRNPA1* significantly increased in the retreat group, while *Dkc1* decreased in controls. *Wrap53* expression also significantly decreased in the retreat group. Wrap53 (also called TCAB1; Freund et al., 2014) is a protein in the Cajal body that binds to telomerase hTR RNA and is important for assembly of the telomerase holoenzyme (Venteicher et al., 2009). Mutations in the *Wrap53* gene cause dyskeratosis congenita, a genetic disease characterized by short telomeres (Zhong et al., 2011). Interestingly, despite the observed decrease in *Wrap53* expression, retreat participants also showed relatively higher expression of multiple components of the TriC complex (*Cct1*, *Cct2*, *Cct3*, *Cct6*, *Cct7*, *Cct8*), which is required for Wrap53 folding. This contrast between *Wrap53* and TriC complex gene expression suggests that retreat participation may lead to dynamic changes in the regulation of telomerase-cofactor folding and telomerase trafficking, which are required for telomerase-mediated telomere elongation.

Retreat participants also showed higher expression levels of *CTC1*, *UPF3A*, *UPF1*, *ATRX*, *ATM*, and *ATR*, which are involved in telomere replication, repair, and maintenance. CTC1 is a component of the CST complex (CTC1-STN1-TEN1), which functions in telomere replication. CTC1 is essential for telomere length maintenance due to its role in mediating C-strand fill-in (Feng, Hsu, Kasbek, Chaiken, & Price, 2017). UPF3A and UPF1 are components of the nonsense-mediated RNA decay pathway (NMD) involved in telomere replication (Lew, Enomoto, & Berman, 1998). UPF1 interacts both with telomerase and the telomeric factor TPP1. UPF1 sustains telomere leading-strand replication, and its depletion leads to telomere instability—owing largely to inefficient telomere leading-strand replication (Chawla et al., 2011). ATRX is a chromatin-remodeling factor that acts on telomeres. It inhibits alternative lengthening of telomeres (ALT)—a telomerase-independent telomere-lengthening pathway—and may, therefore, promote telomerase-dependent telomere length regulation (Clynes et al., 2015; Napier et al., 2015). ATM and ATR are DNA-damage-response protein kinases (PIKK) that phosphorylate shelterin components, and their depletion reduces telomerase complex assembly. They are also required for the recruitment of telomerase to telomeres, making them crucial for telomerase-mediated telomere maintenance (Tong et al., 2015). ATRIP, which increased in retreat participants, interacts with both ATM and ATR (Doksani & Lange, 2015). Collectively, these findings support the conclusion that retreat participants experienced improved telomerase-mediated telomere lengthening or maintenance, despite the lack of observable change in telomerase activity.

There were also differences in the expression of genes involved in the non-homologous end-joining (NHEJ) pathway (*Xrcc3*, *Xrcc4*, *Xrcc6*, and *Xrcc6bp1*). This pathway is implicated in the repair of dysfunctional telomeres, which may otherwise result in telomere fusions

(Doksani & Lange, 2015). Expression of *Xrcc3* and *Xrcc4* decreased in retreat participants, while *Xrcc6* and *Xrcc6bp1* decreased in controls. Interestingly, cells defective in Xrcc4 do not show changes in telomere length or function, which suggests that this protein may not be involved in telomere maintenance (Yasaei & Slijepcevic, 2010); however, *Xrccr4* is a DNA repair gene that shows a linear, age-related increase in expression in human fibroblast from older females (Kalfalah et al., 2015). Thus, its decrease in retreat participants might signal some other mechanism of cell rejuvenation.

Finally, we observed changes in genes coding for OB-fold proteins, which are involved in single-strand DNA binding and telomere protection. Retreat participants showed a relative increase in the expression of *Obfc1* and *Obfc2B/Nabp2*, but a decrease in *Obfc2A/Nabp1*, which is in the same SOSS (sensor of single-strand DNA) complex as OBFC2B. *Pot1*, which is a negative regulator of telomere length (Lei, Podell, Baumann, & Cech, 2003; Lei, Podell, & Cech, 2004; Nandakumar et al., 2012), also decreased in retreat participants.

In sum, these gene expression changes suggest that meditation retreat participation may positively influence various processes related to cellular aging, including telomere maintenance and telomerase assembly and function. This profile of results further suggests that the apparent increases observed in telomere length may have resulted from both telomerase-mediated and telomerase-independent pathways. Future studies will be needed to replicate these findings and to differentiate these mechanisms.

Personality and Telomere Length Changes

In a recent review on personality and physical health, Murray and Booth (2015) reported that conscientiousness is largely associated with better health-related outcomes, and neuroticism with worse health-related outcomes. The authors also found little evidence for a link between

agreeableness and health, and heterogeneous results with regard to extraversion and openness. In the present study, we observed a significant decrease in neuroticism across groups, which was largely driven by changes in retreat participants. However, baseline levels of neuroticism—not changes in neuroticism—positively predicted changes in telomere length across retreat. Neuroticism concerns how easily or often a person becomes upset or distressed, and is closely related to constructs of anxiety and depression (Carver & Connor-Smith, 2010). As such, one interpretation for our results is that residential retreats may afford individuals vulnerable to negative emotional reactivity a stable and supportive environment in which to attend to their ongoing experiences and to develop adaptive coping strategies amongst fewer interpersonal stressors. This combination of factors may slow cellular degradation or promote restoration.

Although telomere length was significantly related to both anxiety and depression overall, neither baseline anxiety nor retreat-related decreases in anxiety predicted changes in telomere length. Despite the conceptual relatedness of neuroticism, anxiety, and depression (all *r*s > .6 in the present sample), our results point to heterogeneity in their consequences for telomeric regulation. For example, neuroticism is thought to index aspects of hostility and impulsiveness unrelated to depression, while depression includes somatic components not captured by personality scales (Luchetti, Barkley, Stephan, Terracciano, & Sutin, 2014). More research will be needed to delineate how the facets of each of these constructs relate to markers of cell aging.

We also provide novel evidence suggesting agreeableness may contribute to health outcomes. Although agreeableness increased across both groups, participants who were lowest in agreeableness at baseline were more likely to show a retreat-related increase in telomere length. Agreeableness tends to modulate interpersonal reactions, such that individuals higher in

agreeableness become less upset over others' transgressions, whereas those low in agreeableness may become more aggressive and antagonistic (Meier and Robinson, 2004; reviewed in Carver and Connor-Smith, 2010). As with individuals high in neuroticism, those low in agreeableness may derive greater telomere-related benefits from the reduced social interaction characteristic of silent retreat.

Finally, while our findings do not support a link between conscientiousness and health, they do support a nuanced relationship between health and openness to experience. High levels of openness moderated changes in telomere length, such that retreat participants showed apparent telomere lengthening, while control participants showed apparent telomere shortening. One possible interpretation is that openness to ones' ongoing experience in a supportive retreat environment may be important for promoting telomere-related benefits, whereas openness to diverse experiences outside of retreat may lead to engagement in both beneficial and health-compromising behaviors.

Limitations and Suggestions for Future Research

Within the Western Insight tradition, residential retreats are used to facilitate periods of concentrated meditation practice (Goldstein & Kornfield, 1987). Our results support the supposition that intensive practice within a supportive environment—under the guidance of experienced teachers and while removed from the usual demands of daily life—may have important implications for psychological and cellular health. The present findings further suggest that individual differences in personality traits may enhance or mitigate some of the cellular benefits of attending retreat. Nevertheless, there are two notable limitations to our study design: lack of group randomization and the lack of a passive control condition. There are inherent challenges in creating randomized, active control interventions for month-long residential

interventions that are structurally comparable in duration, location, diet, teachers, social components, and other potentially contributing factors. We further acknowledge that attending a 1-month residential retreat at a Buddhist-based meditation center is not feasible or appropriate for everyone, limiting the generalizability of our results. For these reasons, we opted to recruit a comparison group designed to minimize discrepancies in meditation experience, expectations, and motivations between groups. Indeed, groups were matched on a number of lifestyle and behavioral-health relevant factors (see Supplemental Materials).

As the present study was not designed to disambiguate the effects of formal meditation practice from the retreat intervention as a whole, it is important to consider our results in tandem with shorter interventions employing more targeted controls, such as Epel et al. (2016), who utilized a vacation control condition to account for the biological consequences of removal from daily life stressors. Although we attribute our results to the retreat intervention overall, the relations we observed between measures of retreat engagement and telomere biology suggest that formal meditation practice related meaningfully to measures of cell aging.

As telomere length varies by cell type, one potential confound that warrants further investigation is the possibility that immune cell distributions may have changed between assessments. We measured telomere length in PBMCs, which consist of many different cell types, including monocytes and lymphocytes (T-cells—such as CD8 and CD4 cells, B-cells, and natural killer cells). Lymphocyte redistribution, which can occur temporarily in response to acute stress (Dhabhar, 2011; Dhabhar, Malarkey, Neri, & McEwen, 2012; Rosenberger et al., 2009) may lead to "pseudo-lengthening," whereby average telomere length measurements increase due to an increase in cell types having longer telomeres (Epel, 2012). It is possible that meditation training may have reduced retreat participants' stress reactivity, thereby minimizing stress-

related cell redistribution during the Time 2 blood-collection. However, we sought to minimize the influence of acute stress responses for controls in our protocol by incorporating a period of meditation prior to the blood draw. It is also possible that the retreat intervention reduced overall psychological stress profiles, thereby shifting cell populations in a more lasting manner, as MBSR has been shown to increase or maintain CD4+ T cells in HIV patients (Creswell et al., 2009; Alinaghi et al., 2012). There are many ways that changes in cell types could produce a pattern of telomere elongation, and because we did not measure per-cell telomere length changes, we label the phenomenon here "apparent telomere lengthening," and await further evidence of true per cell telomere lengthening in humans. However, the pattern of gene expression changes observed in the present study bolsters the conclusion that the retreat-related telomere increases are attributable to actual, versus pseudo, lengthening. Future studies using flow cytometry to assess cell distributions will be necessary to disambiguate these alternatives.

Conclusions

In sum, we report that meditation practice in a retreat context may have salutary effects on telomere regulation, which appear sensitive to individual differences in personality. As this is the first intervention to demonstrate an apparent increase in telomere length in such a short timeframe, this finding should be interpreted both with enthusiasm and caution. Future work will be needed to replicate these relatively short-term effects and to determine whether increases in telomere length persist once practitioners resume their typical daily lives and normative social engagement. We also found provocative relationships between telomerase activity and measures of retreat engagement and prior meditation experience, and propose an explanatory framework that might reconcile these and other telomerase findings in the meditation research literature.

Finally, we demonstrated the utility of assessing multiple measures of telomere biology and statistically modeling sources of variability in telomere length telomere length assays.

TABLES

Table 1.1

Descriptive Statistics for Telomere Length, Telomerase Activity, and Self-report Measures

	Time 1					Time 2				
		Control		Retreat			Control		Retreat	
	Alpha	*n*	Mean (*SD*)	*n*	Mean (*SD*)	Alpha	*n*	Mean (*SD*)	*n*	Mean (*SD*)
Telomere length (t/s)		132	1.11 (0.19)	106	1.16 (0.24)		110	1.10 (0.19)	97	1.22 (0.25)
Telomerase activity		32	10.57 (7.86)	28	8.26 (6.73)		27	9.09 (7.73)	26	6.21 (3.08)
Personality (BFI; 1-7)										
Neuroticism	0.90	30	3.4 (1.4)	26	3.6 (1.2)	0.89	27	3.1 (1.4)	25	3.5 (1.1)
Agreeableness	0.71	30	5.2 (0.9)	26	5.5 (0.5)	0.77	27	5.4 (0.9)	25	5.7 (0.6)
Conscientiousness	0.85	30	5.5 (1.0)	26	5.5 (1.0)	0.86	27	5.3 (1.0)	25	5.6 (09)
Openness	0.80	30	5.5 (0.8)	26	5.5 (0.7)	0.78	27	5.6 (0.8)	25	5.6 (0.8)
Extraversion	0.81	30	4.5 (1.1)	26	4.3 (1.0)	0.79	27	4.6 (0.8)	25	4.3 (1.0)
Trait anxiety (STAI; 1-4)	0.94	30	38.3 (11.3)	28	38.2 (9.4)	0.94	27	38.0 (12.0)	25	35.2 (6.7)
Depression (CES-D; 0-3)	0.89	30	13.0 (9.1)	27	13.5 (8.4)	0.88	27	13.9 (11.1)	25	13.5 (6.9)

Note: Telomere length means represent unweighted group means. Due to variability in the number of available t/s ratios across participants, these values will differ slightly from the model means represented elsewhere. For telomere length, *n* corresponds to the number of t/s values (across runs) used in multilevel analyses; for all other measures *n* corresponds to the number of participants. The number of participants for telomere length is equivalent to the number of subjects for whom telomerase values are available at each assessment. Converting t/s ratios to base pairs [bp = 3274+2413*(t/s)] and then dividing by 1000 yields the following mean base pair estimates: Control = 5.95 kilo base pairs (kbp) at Time 1 and 5.93 kbp at Time 2; Retreat = 6.07 kbp at Time 1 and 6.22 kbp at Time 2.

Table 1.2

Estimated Mean Differences in Gene Expression by Group and Assessment: Significant Discoveries

Gene	Summary of discoveries		*p*-value, uncorrected				Estimated mean difference (*SE*)			
	Change over time	Group difference	Time (Post - Pre)		Group (Control - Retreat)		Time (Post - Pre)		Group (Control - Retreat)	
			Retreat	Control	Pre	Post	Retreat	Control	Pre	Post
Atrip	R↑		.011	.054	.692	.222	0.412 (0.157)	0.276 (0.140)	-0.059 (0.148)	-0.194 (0.158)
Cct1	R↑	Post (R > C)	.003	.609	.139	< .001	0.259 (0.082)	0.039 (0.076)	-0.121 (0.081)	-0.341 (0.086)
Cct6	R↑	Post (R > C)	< .001	.052	.389	.001	0.605 (0.138)	0.252 (0.127)	-0.118 (0.137)	-0.471 (0.144)
Gar1	R↑		.010	.028	.588	.247	0.429 (0.160)	0.322 (0.143)	-0.083 (0.154)	-0.190 (0.163)
Hnrnpa1	R↑		.005	.021	.085	.022	0.518 (0.179)	0.394 (0.165)	-0.309 (0.178)	-0.434 (0.187)
Atmin	R↑, C↑		< .001	.006	.857	.233	0.725 (0.202)	0.513 (0.181)	-0.035 (0.194)	-0.248 (0.207)
Nhp2	R↑, C↑		.004	.005	.399	.244	0.353 (0.119)	0.309 (0.106)	-0.094 (0.111)	-0.139 (0.118)
Ctc1	R↑, C↓	Post (R > C)	.012	.016	.197	.002	0.425 (0.164)	-0.364 (0.147)	0.222 (0.171)	-0.567 (0.178)
Nabp2	R↑, C↓	Post (R > C)	.002	< .001	.112	< .001	0.157 (0.050)	-0.172 (0.046)	-0.142 (0.088)	-0.471 (0.090)
Nabp1	R↓	Post (C > R)	< .001	.075	.442	< .001	-0.257 (0.036)	-0.061 (0.034)	0.038 (0.049)	0.233 (0.050)
Pot1	R↓		.017	.036	.931	.670	-0.124 (0.050)	-0.097 (0.045)	-0.004 (0.050)	0.023 (0.054)
Wrap53	R↓		.006	.057	.842	.495	-0.176 (0.061)	-0.106 (0.055)	-0.015 (0.076)	0.055 (0.080)
Xrcc3	R↓		.017	.023	.577	.796	-0.123 (0.050)	-0.104 (0.045)	-0.036 (0.065)	-0.018 (0.068)
Xrcc4	R↓	Post (C > R)	< .001	.443	.242	.014	-0.188 (0.053)	0.038 (0.049)	-0.070 (0.060)	0.156 (0.063)
Tep1	R↓, C↓		.003	.008	.746	.437	-0.138 (0.045)	-0.112 (0.040)	0.017 (0.054)	0.044 (0.057)
Tinf2	R↓, C↓		.006	.016	.588	.993	-0.131 (0.046)	-0.103 (0.041)	-0.027 (0.050)	0.000 (0.053)
Upf1	R↓, C↓	Post (R > C)	.011	< .001	.282	.006	-0.117 (0.044)	-0.209 (0.041)	-0.054 (0.050)	-0.146 (0.053)
Atm	C↓	Post (R > C)	.132	.004	.247	< .001	0.086 (0.056)	-0.157 (0.052)	-0.069 (0.060)	-0.313 (0.063)
Atr	C↓	Post (R > C)	.078	.014	.965	.003	0.064 (0.035)	-0.083 (0.033)	0.002 (0.045)	-0.145 (0.047)
Atrx	C↓	Post (R > C)	.559	.001	.489	.013	-0.023 (0.039)	-0.124 (0.036)	-0.036 (0.052)	-0.138 (0.054)
Cct2	C↓	All (R > C)	.268	.012	.003	< .001	0.059 (0.053)	-0.127 (0.049)	-0.186 (0.061)	-0.373 (0.063)
Cct7	C↓	Post (R > C)	.163	.005	.282	< .001	0.089 (0.063)	-0.167 (0.058)	-0.068 (0.063)	-0.324 (0.066)
Cct8	C↓	Post (R > C)	.235	.003	.106	< .001	0.071 (0.059)	-0.167 (0.055)	-0.148 (0.091)	-0.386 (0.093)
Dkc1	C↓		.512	< .001	.430	.086	-0.027 (0.041)	-0.143 (0.037)	0.035 (0.044)	-0.081 (0.047)
Obfc1	C↓	Post (R > C)	.044	.017	.069	.013	0.204 (0.099)	-0.220 (0.089)	0.175 (0.095)	-0.249 (0.099)
Upf3a	C↓		.204	< .001	.992	.034	-0.096 (0.075)	-0.323 (0.069)	-0.001 (0.102)	-0.228 (0.106)
Xrcc6	C↓	Post (R > C)	.361	< .001	.557	< .001	0.039 (0.042)	-0.137 (0.039)	-0.029 (0.049)	-0.205 (0.051)
Xrcc6bp1	C↓		.815	< .001	.636	.023	-0.011 (0.045)	-0.247 (0.042)	0.039 (0.083)	-0.197 (0.085)
Cct3		Post (R > C)	.033	.061	.053	< .001	0.143 (0.065)	-0.116 (0.060)	-0.159 (0.081)	-0.418 (0.085)

Table 1.2 Continued

Gene	Summary of discoveries		*p*-value, uncorrected				Estimated mean difference (*SE*)			
	Change over time	Group difference	Time (Post - Pre)		Group (Control - Retreat)		Time (Post - Pre)		Group (Control - Retreat)	
			Retreat	Control	Pre	Post	Retreat	Control	Pre	Post
Cct4		Pre (C > R)	.273	.021	.008	.084	-0.052 (0.047)	-0.100 (0.042)	0.152 (0.056)	0.104 (0.059)
Hmbox1		Post (R > C)	.028	.053	.304	< .001	0.124 (0.055)	-0.101 (0.051)	-0.093 (0.090)	-0.317 (0.092)
Smg5		Post (R > C)	.088	.150	.124	< .001	0.100 (0.057)	-0.078 (0.053)	-0.134 (0.086)	-0.311 (0.089)
Terf1		Pre (C > R)	.184	.216	.010	.995	0.192 (0.143)	-0.160 (0.129)	0.351 (0.134)	-0.001 (0.139)
Terf2		All (R > C)	.024	.582	.001	< .001	0.083 (0.036)	-0.018 (0.033)	-0.221 (0.065)	-0.323 (0.067)
Upf2		Post (R > C)	.061	.131	.455	.001	0.088 (0.046)	-0.064 (0.042)	-0.041 (0.055)	-0.193 (0.058)
Upf3b		All (R > C)	.184	.720	.015	< .001	0.096 (0.071)	-0.024 (0.066)	-0.188 (0.076)	-0.308 (0.080)
Xrn1		All (R > C)	.660	.346	.001	< .001	-0.019 (0.044)	-0.039 (0.041)	-0.216 (0.065)	-0.235 (0.067)

Note: Statistical significance was assessed at the FDR-adjusted threshold of $\alpha = .018$ for log-transformed gene expression levels. Change over time indicates significant increases (↑) or decreases (↓) in the Retreat (R) or Control (C) group across assessments. Pre indicates a group difference at Time 1, Post at Time 2, and All at both assessments. R>C = retreat group greater than controls. Estimated mean differences for genes showing no significant discoveries are presented in Table S6.

Table 1.3

Changes in Telomere Length and Telomerase Predicted by Neuroticism and Agreeableness

	Change in telomere length		Change in telomerase	
Estimate	Neuroticism	Agreeableness	Neuroticism	Agreeableness
Intercept	0.048 (0.015)**	0.067 (0.015)***	-0.345 (0.139)	-0.207 (0.185)
Personality at pre-assessment	0.042 (0.013)**	-0.082 (0.027)**	0.407 (0.126)**	-0.345 (0.322)
Personality change	0.041 (0.023)	-0.048 (0.034)	-0.196 (0.214)	0.320 (0.409)
Fit statistics				
-2 log likelihood	-44.6	-47.5	51.4	57.3
AIC	-42.6	-45.5	51.6	59.3
BIC	-41.6	-44.5	52.4	60.3

Note: Models predicting changes (Time 2 – Time 1) in telomere length and telomerase from baseline (Time 1) personality and changes in personality. Personality predictors are indicated by column subheadings. * $p \leq 0.25$, ** $p \leq 0.01$, *** $p \leq 0.001$.

FIGURES

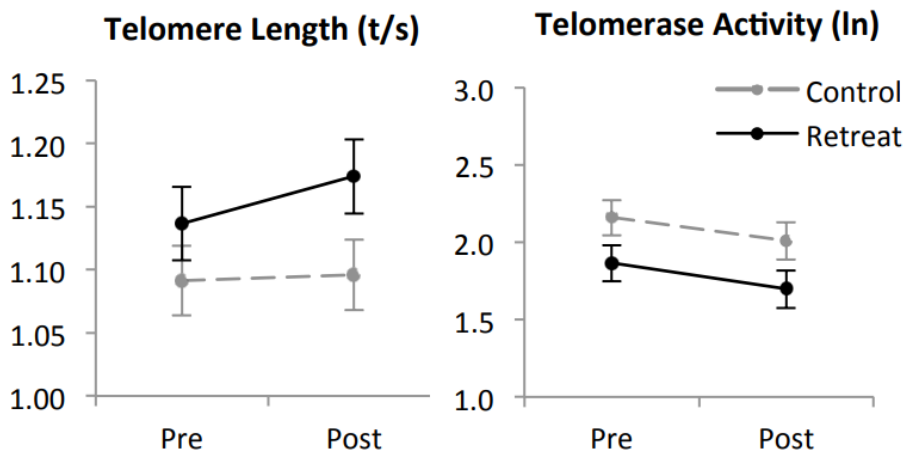

Figure 1.1: Change in telomere length and telomerase by group. Error bars represent standard error.

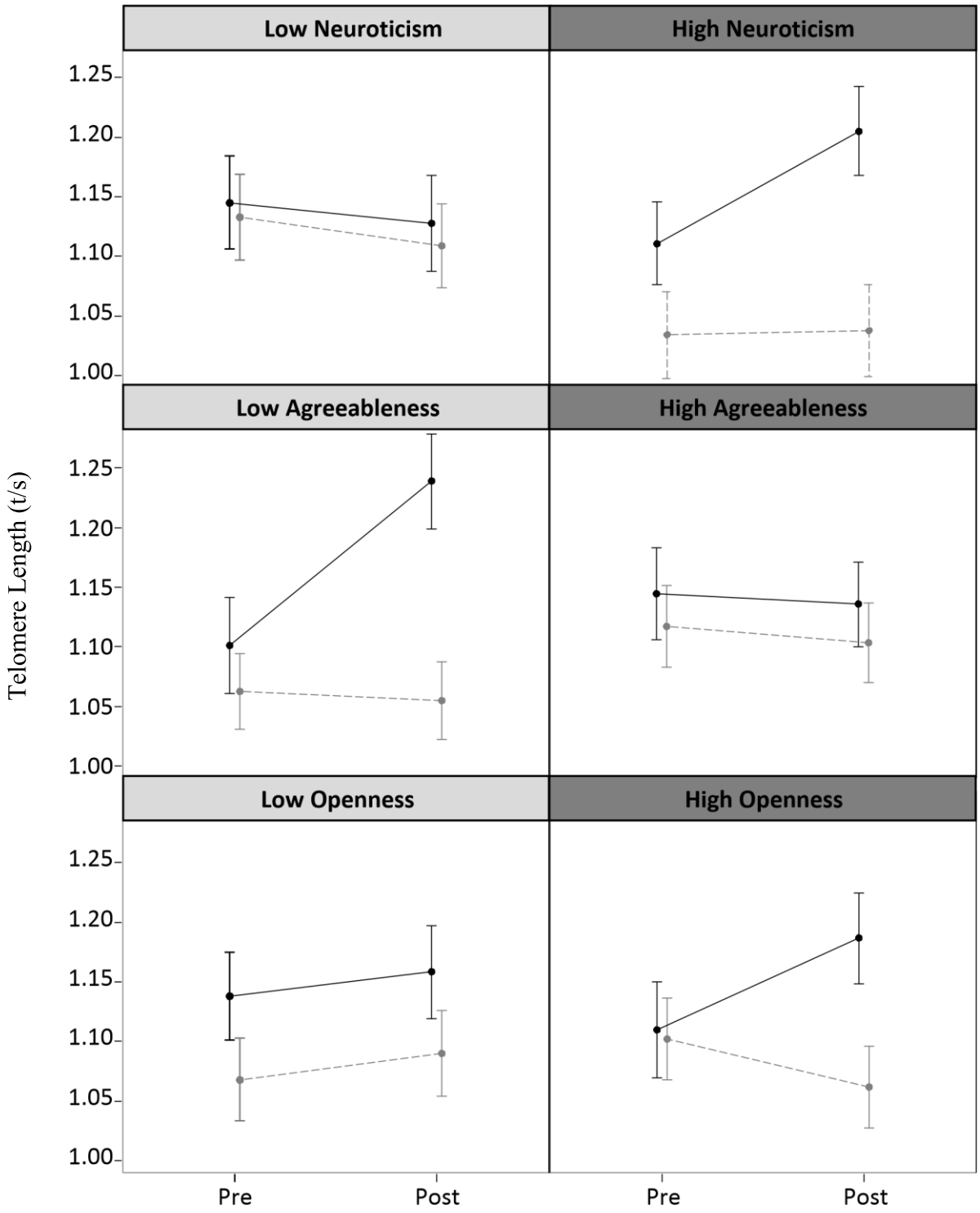

Figure 1.2. Simple slopes for personality effects on telomere length. Model estimates are plotted for 1 SD below (Low) and 1 SD above (High) the mean of the personality variable. Error bars represent the standard error. Black solid lines represent the retreat group, and grey dashed lines represent controls.

APPENDIX

Chapter 1 Supplementary Material

Group differences in baseline participant characteristics were assessed using independent *t*-tests, Wilcoxon rank sums, or chi-square tests where appropriate (Table S1). The groups were well educated on average. When asked about their highest level of completed education, 42.1% of retreat participants and 47.1% of comparison participants reported holding graduate or professional degrees; 10.7% and 20.6%, respectively, reported having attended some graduate or professional schooling; 32.1% of retreat participants and 20.6% of comparison participants reported holding college diplomas with no graduate or professional schooling; and the remaining 14.29% and 8.82%, respectively, reported having attended some college with no earned degree. Annual household income ranged from "$10,000 or less" to "$100,000 or more." The median income category for each group was $40,001–$50,000 annually.

Of the retreat group, 67.9% of participants reported their sexual orientation as heterosexual, 7.1% as bisexual, 10.7% as gay or lesbian, 4.8% as a different orientation, and 3.8% declined to state an orientation. Of the comparison group, 79.4% of participants identified as heterosexual, 5.9% as bisexual, 2.9% as gay or lesbian, and 8.8% declined to state or did not report. Participants were invited to endorse as many of the following marital categories as they felt appropriate (thus percentages may exceed 100% across categories): Single (Retreat group [R] = 42.9%, Comparison group [C] = 50.0%); Dating one person (R = 17.9%, C = 17.7%); Dating a number of people (R = 3.6%, C = 5.9%); Cohabitating (R = 10.7%, 8.8%); Married (R = 25.0%, C = 20.6%); Divorced (R = 21.4%, C = 14.7%); and Widowed (C = 2.9%). Participants were further asked whether they identified as religious (R = 46.4%, C = 50.0%), non-religious (R = 53.6%, C = 38.2%), or declined to state (C = 8.8%). Of those who identified as religious,

participants endorsed the following religious affiliations: Buddhist (R=12, C = 15), Jewish (R = 1, C = 1), or multi-faith (C = 1).

Individual differences in telomere length and telomerase activity levels may be associated with a number of lifestyle factors; therefore, we verified that participant groups were matched on dimensions of the Lifestyle Profile II (Walker et al., 1987) at pre-assessment (Table S1), including health responsibility, physical activity, nutrition, spiritual growth, interpersonal relations, and stress management. Health responsibility was significantly correlated with BMI at pre-assessment, $r(26) = -.41$, $p = .032$, whereas interpersonal relations were significantly correlated with BMI at post-assessment, $r(23) = -.61$, $p = .001$. Spiritual growth was correlated with telomere length at post-assessment, $r(23) = .42$, $p = .037$.

Participants were asked to estimate their prior meditation experience using several metrics, including the number of years they had practiced meditation; the amount of time they spent practicing at home; and the length and number of retreats they had attended in their lifetime and over the previous year (See Table S2). At post-assessment, retreat participants were also asked to estimate the number of hours per week they had spent in formal meditation practice *during* the study intervention ($n = 25$, $M = 52.83$, $SD = 15.9$, range = 14–77), and to indicate the degree to which they felt they had practiced more diligently than their peers ($n = 24$, $M = 2.79$, $SD = 1.41$, range = 1–6; on a scale ranging from 1 [strongly disagree] to 7 [strongly agree]). These post-retreat measures of engagement were strongly correlated with one another, Spearman $r_s(22) = .61$, $p = .002$. Perceived diligence was also correlated with a number of estimates of prior meditation experience, including overall lifetime hours of practice, $r_s(22) = .51$, $p = .011$, lifetime hours of home practice, $r_s(22) = .67$, $p < .001$, total number of retreats attended, $r_s(22) = .47$, $p = .021$, longest retreat in days, $r_s(21) = .80$, $p < .0001$, and total number of days spent on

retreat over their lifetime, $r_s(22) = .70$, $p < .001$. Thus, participants' reports of perceived practice diligence during the intervention appear to be a meaningful index of overall practice engagement.

As reported in the accompanying manuscript, retreat participants' perceived diligence was positively related to changes in telomere length across retreat, while telomerase levels at post-assessment were negatively related both to diligence and to estimated hours of practice during the intervention. Reports of perceived diligence were also negatively related to baseline agreeableness, $r_s(21) = -0.49$, $p = .016$, suggesting that retreat participants who practiced more diligently were less agreeable at the onset of retreat, incurred the greatest increases in telomere length, and showed lower levels of telomerase after three weeks of practice. Additionally, for retreat participants, telomerase at post-assessment was negatively correlated with number of lifetime practice hours, $r(23) = -.40$, $p = .048$, and length of longest retreat attended (in days), $r(23) = -.74$, $p < .0001$. Longest retreat attended was also inversely related to change in telomerase, $r(23) = -.42$, $p = .039$, such that retreat participants who had previously attended longer retreats (e.g., 1–3 months) showed greater declines in telomerase from pre- to post-assessment. All correlations remained significant after partialling for effects of age. Taken together, these correlations suggest that recent and long-term practice engagement are related to lower levels of telomerase at the end of retreat. When considered alongside observed increases in telomere length in these same participants, these patterns suggest that practitioners with greater experience—or who are able to engage in more practice time on retreat—may derive greater restorative benefits during the retreat, resulting in increased telomere length and, potentially, lesser need for the reparative functions of telomerase. This reasoning is consistent with findings

from Epel et al. (2016) who show that experienced meditators had the lowest levels of telomerase at baseline, compared to meditation-novice and vacation control groups.

Table S1 *Group demographics*

	Comparison group					Retreat group					Test statistic		
	n	Mean	*SD*	Min	Max	*n*	Mean	*SD*	Min	Max	Statistic	*df*	*p*
Age	34	51.94	12.89	27	69	28	49.29	12.61	25	68	0.82	60	.418
BMI at pre-assessment	34	24.58	3.63	20	35	28	23.64	3.98	18	34	0.97	60	.335
Education	33	5.09	1.04	3	6	28	4.82	1.16	3	6	-0.87	—	.383
Annual household income	33	6.21	3.38	1	11	28	6.00	3.64	1	11	-0.25	—	.804
Lifestyle factor	30	2.53	0.60	1.44	3.67	28	2.40	0.52	1.33	3.44	0.87	56	.387
Health responsibility	30	2.91	0.47	1.63	3.75	28	2.92	0.55	1.75	3.88	-0.10	56	.921
Physical activity	30	2.94	0.41	2.11	3.44	28	2.90	0.43	2.11	3.67	0.33	56	.743
Nutrition	30	3.18	0.50	2.22	3.89	28	3.21	0.60	2.00	4.00	-0.24	56	.813
Spirituality	30	3.21	0.57	2.22	4.00	28	3.28	0.61	1.67	4.00	-0.43	56	.668
Stress	30	3.08	0.51	1.88	3.88	28	3.09	0.45	1.88	4.00	-0.07	56	.948

Note: Highest level of educational achievement was coded on the following scale: 1 = less than high school diploma, 2 = high school diploma, 3 = some college, 4 = college degree, 5 = some graduate study, 6 = graduate degree. Annual household income was coded on the following scale 1 = less than $10,000, 2 = $10,001–$20,000, 3 = $20,001–$30,000, 4 = $30,001–$40,000, 5 = $40,001–$50,000, 6 = $50,001–$60,000, 7 = $60,001–$70,000, 8 = $70,001–$80,000, 9 = $80,001–$90,000, $90,001–$100,000, 11 = more than $100,000. *T*-tests are reported for age and BMI, and nonparametric Wilcoxon rank sum tests are reported for education and income.

Table S2 *Prior meditation experience*

	Comparison Group					Retreat Group					Test statistic		
	n	Mean	*SD*	Min	Max	*n*	Mean	*SD*	Min	Max	*t*	*df*	*p*
Lifetime experience													
Years of meditation experience	32	14	11.0	1	40	27	11	8.6	1	32	1.22	57	.227
Total lifetime hours of practice	29	4942	4820.0	350	19872	26	4892	4712.0	216	16432	0.04	53	.970
Retreat practice													
Total number of retreats attended	32	12	9.4	1	33	27	20	24.1	2	100	*-1.53*	*33*	*.135*
Longest retreat attended (in days)	32	29	31.6	6	90	26	37	32.8	7	90	-0.93	56	.355
Total days of retreat attended	28	141	163.5	7	684	27	210	243.8	12	900	*-1.23*	*45*	*.226*
Retreat days over the past year	30	10	10.2	0	35	27	28	35.1	0	135	*-2.54*	*30*	***.016***
Home practice													
Lifetime hours of home practice	29	3398	3917.0	120	16872	26	2410	2447.0	40	8232	*1.13*	*48*	*.263*
Average hours per week (past year)	29	4	2.5	0	12	26	6	4.8	0	20	*-1.47*	*36*	*.151*
Average hours per year (past year)	28	217	128.2	10	650	26	242	145.0	20	500	-0.66	52	.511

Note: Test statistics given in italics did not meet equality of variance assumptions; in these instances, the Satterthwaite approximation for degrees of freedom was applied. Sample size (*n*) differs between measures because some participants declined to answer select questions regarding meditation experience.

Table S3 *Real time PCR assay probes*

Telomere-related genes	Name of probe
ATM	Hs01112355_g1
ATMIN	Hs00796220_s1
ATR	Hs00992123_m1
ATRIP	Hs04335019_s1
ATRX	Hs00230877_m1
CCT1	Hs01053946_g1
CCT2	Hs00197562_m1
CCT3	Hs00195623_m1
CCT4	Hs00272345_m1
CCT5	Hs00603745_mH
CCT6A	Hs00798979_s1
CCT7	Hs00362446_m1
CCT8	Hs00607229_mH
CTC1	Hs01558646_m1
DKC1	Hs00154737_m1
GAR1, dyskerin-associated	Hs00852376_g1
HMBOX1, aka HOT1 telomere protein	Hs00226156_m1
HNRNPA1	Hs01656228_s1
NAF1	Hs00370696_m1
NHP2	Hs00950764_g1
NOP10	Hs00430282_m1
NOP14, dyskerin-associated	Hs00379582_m1
OBFC1	Hs00372796_m1
OBFC2A	Hs00224567_m1
OBFC2B	Hs00225302_m1
PINX1	Hs00363228_mH
POT1	Hs00209984_m1
RTEL1	Hs01566915_m1
SMG5 EST1B, telomerase component	Hs00383399_m1
SMG6 EST1A, telomerase-binding protein	Hs00214019_m1
SMNDC1	Hs01090302_m1
TEP1	Hs00200091_m1
TERC	Hs03454202_s1
TERF1	Hs00819517_mH
TERF2	Hs00194619_m1
TERF2IP	Hs00430292_m1
TERT	Hs00972656_m1
TINF2	Hs01554309_g1
TNKS	Hs00186671_m1
TPP1	Hs00166099_m1
UPF1	Hs00161289_m1
UPF2	Hs00210187_m1
UPF3A	Hs00425159_g1
UPF3B	Hs00224875_m1
WRAP53	Hs00216360_m1
XRCC1	Hs00959834_m1
XRCC2	Hs03044154_m1
Telomere-related genes	Name of probe

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XRCC3	Hs00193725_m1
XRCC4	Hs00243327_m1
XRCC5 Ku86	Hs00897854_m1
XRCC6 Ku70	Hs01922655_g1
XRCC6BP1	Hs00384755_m1
XRN1	Hs00404871_m1
XRN2	Hs01071302_m1

Table S4 *Stepwise models of telomere length*

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8
Fixed effects								
Intercept	1.143 (0.010)***	1.127 (0.026)***	1.117 (0.029)***	1.085 (0.037)***	1.096 (0.032)***	1.123 (0.04)***	1.091 (0.039)***	1.134 (0.036)***
Group (ref. = Control)	—	—	—	0.052 (0.051)	0.027 (0.042)	0.064 (0.05)	0.052 (0.064)	0.024 (0.056)
Time (ref. = Time 1)	—	—	—	-0.003 (0.009)	-0.003 (0.009)	-0.003 (0.009)	-0.003 (0.009)	-0.003 (0.009)
Group × Time	—	—	—	0.046 (0.013)***	0.046 (0.013)***	0.049 (0.014)***	0.045 (0.014)***	0.048 (0.014)***
Age	—	—	—	—	-0.009 (0.002)***	—	—	-0.010 (0.002)***
Gender	—	—	—	—	—	-0.119 (0.051)*	—	-0.116 (0.048)*
BMI at Time 1	—	—	—	—	—	—	-0.019 (0.007)**	-0.006 (0.006)
BMI change	—	—	—	—	—	—	0.002 (0.055)	-0.041 (0.047)
Variance components								
Residual variance	0.048 (0.003)	0.006 (0.000)	0.005 (0.000)	0.005 (0.000)***	0.005 (0.000)***	0.005 (0.000***	0.005 (0.000)***	0.005 (0.000)***
Participant intercept	—	0.040 (0.007)	0.039 (0.007)***	0.038 (0.007)***	0.025 (0.005)***	0.035 (0.007)***	0.036 (0.007)***	0.024 (0.005)***
Assay run intercept	—	—	0.002 (0.001)	0.002 (0.001)	0.002 (0.001)	0.002 (0.001)	0.002 (0.001)	0.002 (0.001)
Fit statistics								
-2 log likelihood	-83.2	-764.4	-837.6	-839.6	-853.7	-821.1	-774.5	-763.6
AIC	-81.2	-760.4	-831.6	-833.6	-847.7	-815.1	-768.5	-757.6
BIC	-77.1	-756.2	-837.6	-839.6	-853.7	-821.1	-774.5	-763.6

Note: Model 1 is an empty means model. Models 2 and 3 test for random effects of participants and assay runs, respectively. Model 4 tests the interaction of group and time. Models 5–8 test for covariates of age, gender, and BMI. For all models, fixed effects and variance components are restricted maximum likelihood estimates, with standard error in parentheses. Ref. indicates the reference category for categorical predictors. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Table S5 *Telomere length predicted by anxiety, depression, and personality*

		Self-report models				
	Base model	Anxiety	Depression	Neuroticism	Agreeableness	Openness
Fixed effects						
Intercept	1.140 (0.033)***	1.143 (0.033)***	1.139 (0.033)***	1.139 (0.033)***	1.145 (0.033)***	1.140 (0.034)***
Age	-0.009 (0.002)***	-0.011 (0.002)***	-0.011 (0.002)***	-0.010 (0.002)***	-0.010 (0.002)***	-0.010 (0.002)***
Gender	-0.135 (0.040)**	-0.136 (0.039)***	-0.141 (0.038)***	-0.143 (0.040)***	-0.142 (0.039)***	-0.140 (0.041)**
Group (ref. = Control)	0.048 (0.039)	0.049 (0.039)	0.042 (0.038)	0.045 (0.040)	0.033 (0.039)	0.038 (0.040)
Time (ref. = Pre)	-0.003 (0.009)	-0.004 (0.010)	-0.008 (0.01)	-0.010 (0.011)	-0.011 (0.010)	-0.009 (0.010)
Time × Group	0.049 (0.014)***	0.048 (0.016)**	0.053 (0.015)***	0.048 (0.015)**	0.075 (0.015)***	0.058 (0.015)***
Self-report	—	-0.005 (0.002)**	-0.004 (0.002)*	-0.039 (0.015)*	0.035 (0.018)*	0.021 (0.020)
Self-report × Group	—	0.003 (0.002)	0.002 (0.002)	0.025 (0.020)	-0.007 (0.035)	-0.040 (0.032)
Self-report × Time	—	0.001 (0.001)	0.000 (0.001)	0.011 (0.007)	-0.004 (0.011)	-0.039 (0.013)**
Self-report × Time × Group	—	0.001 (0.002)	0.003 (0.002)	0.033 (0.013)*	-0.091 (0.024)***	0.075 (0.019)***
Variance components						
Participant intercept	0.021 (0.004)***	0.019 (0.004)***	0.019 (0.004)***	0.020 (0.004)***	0.019 (0.004)***	0.021 (0.004)***
Assay run intercept	0.002 (0.001)	0.002 (0.001)*	0.002 (0.001)*	0.001 (0.001)	0.002 (0.001)	0.001 (0.001)
Residual variance	0.005 (0.000)***	0.005 (0.000)***	0.005 (0.000)***	0.005 (0.000)***	0.005 (0.000)***	0.005 (0.000)***
Fit statistics						
-2 log likelihood	-839.3	-726.4	-720.6	-737.0	-744.8	-733.3
AIC	-833.3	-720.4	-714.6	-731.0	-738.8	-727.3
BIC	-839.3	-726.4	-720.6	-737.0	-744.8	-733.3

Note: The base model for telomere length includes experimental design variables and covariates of age and gender, as determined through the model-building procedure given in Table S4. Self-report models include main effects and interactions with self-report predictors as indicated by the column headings. For all models, fixed effects and variance components are restricted maximum likelihood estimates, with standard error in parentheses. Ref. indicates the reference category for categorical predictors. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Table S6 *Estimated mean differences in gene expression by group and assessment: non-significant discoveries*

	p-value, uncorrected				Estimated Mean Difference (*SE*)			
	Time (Post − Pre)		Group (Control − Retreat)		Time (Post − Pre)		Group (Control − Retreat)	
Gene	Retreat	Control	Pre	Post	Retreat	Control	Pre	Post
Cct5	.820	.036	.699	.300	-0.013 (0.056)	-0.107 (0.050)	0.025 (0.064)	-0.070 (0.067)
Naf1	.308	.092	.088	.778	-0.044 (0.043)	0.065 (0.038)	-0.093 (0.054)	0.016 (0.056)
Nop10	.024	.337	.789	.177	-0.113 (0.049)	-0.042 (0.044)	0.016 (0.061)	0.087 (0.064)
Nop14	.031	.132	.583	.860	-0.108 (0.049)	-0.066 (0.043)	-0.031 (0.056)	0.010 (0.059)
Pinx1	.089	.527	.981	.038	0.068 (0.039)	-0.022 (0.035)	-0.001 (0.041)	-0.092 (0.044)
Rtel1	.487	.226	.066	.038	0.056 (0.079)	0.087 (0.071)	0.158 (0.085)	0.190 (0.090)
Smg6	.653	.414	.495	.823	0.028 (0.063)	-0.046 (0.056)	0.056 (0.081)	-0.019 (0.085)
Smndc1	.863	.265	.814	.418	0.012 (0.068)	-0.068 (0.061)	0.017 (0.073)	-0.063 (0.077)
Terc	.274	.783	.881	.370	0.071 (0.064)	0.016 (0.058)	-0.010 (0.069)	-0.066 (0.073)
Terf2ip	.766	.062	.293	.431	0.014 (0.047)	-0.079 (0.042)	0.052 (0.049)	-0.041 (0.052)
Tert	.495	.311	.551	.426	-0.125 (0.182)	-0.167 (0.163)	-0.109 (0.182)	-0.151 (0.189)
Tnks	.075	.189	.055	.189	-0.132 (0.073)	-0.087 (0.065)	-0.162 (0.083)	-0.116 (0.088)
Tpp1	.048	.141	.521	.229	-0.138 (0.068)	-0.091 (0.061)	0.048 (0.074)	0.095 (0.078)
Xrcc1	.089	.294	.493	.905	-0.153 (0.088)	-0.083 (0.079)	-0.058 (0.085)	0.011 (0.090)
Xrcc2	.561	.536	.433	.464	-0.091 (0.156)	-0.087 (0.140)	-0.123 (0.156)	-0.119 (0.161)
Xrcc5	.167	.781	.045	.676	-0.066 (0.047)	0.012 (0.042)	-0.100 (0.049)	-0.022 (0.052)
Xrn2	.228	.146	.801	.729	-0.045 (0.037)	-0.050 (0.034)	-0.013 (0.051)	-0.018 (0.053)

Note: This is a continuation of Table 2 of the manuscript, which reports mean differences for genes showing significant discoveries. Genes showing no significant changes across assessments or between groups are reported here. Statistical significance was assessed at the FDR-adjusted threshold of $\alpha = .018$ for log-transformed gene expression levels.

Daily Retreat Schedule

5:15 Wake-up Bell
5:45 Sitting Meditation or Qigong/Sit in upper walking hall

6:45 BREAKFAST
7:30 Work Meditation[1] / Walking Meditation
8:30 Sitting Meditation with Instructions
9:30 Walking Meditation
10:15 Sitting Meditation
11:00 Walking Meditation
11:30 Sitting Meditation

12:15 LUNCH

2:15 Sitting Meditation
3:00 Walking Meditation or Qigong
3:45 Sitting Meditation
4:30 Walking Meditation
5:00 Sitting Meditation w. Brahma Vihara Instructions

5:30 EVENING MEAL

6:45 Sitting Meditation
7:25 Break
7:35 Dharma Talk
8:30 Walking Meditation
9:00 Sitting Meditation /Evening Chanting
9:40 Sleep or Further Practice

[1] Vacuuming, sweeping, dishes, etc.

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Chapter 2

Serum BDNF predicts retreat-related increases in telomere length during a month-long, residential Insight meditation retreat

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ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) is a modulatory neuroprotein implicated in learning, memory, and neuroplasticity. Studies have found increased peripheral BDNF in participants of intensive meditation retreats, indicating that BDNF may play a role in the learning and habit formation that occurs with meditation training. In this study, we assessed changes in BDNF across a month-long Insight meditation retreat. We also explored the relationship between BDNF levels and retreat-related changes in telomerase and telomere length, which are implicated in cell aging and longevity. **Method:** All participants were experienced meditators. Retreat group participants (n = 28) were assessed at the beginning of, and three weeks into, a silent residential retreat at Spirit Rock Meditation Center. Control group participants (n = 34), who were similar in age, gender, and meditation experience, were also assessed across a 3-week interval. BDNF was measured in serum. Telomere length and telomerase activity were measured in PBMCs. **Results:** In retreat participants, higher levels of BDNF at Time 1 predicted telomere lengthening during the retreat. We also found negative correlations between BDNF and telomerase at both time points, and between individual changes in BDNF and telomerase in the retreat group. However, contrary to our prediction, we observed no mean-level change in BDNF in either group. **Conclusion:** Our results suggest that basal BDNF levels may play a role in retreat-related improvements observed in telomere biology.

***Keywords*:** meditation; retreat; BDNF; telomere length, telomerase

INTRODUCTION

Meditation and mindfulness practices have gained interest in western psychology and neuroscience as methods for improving mental and physical health. Clinical studies suggest that meditation-based interventions may be useful in treating anxiety and depression, as well as mitigating the deleterious consequences of stress[5] (Boyd, Lanius, & McKinnon, 2018; Green & Kinchen, 2021; Hoge et al., 2021). Research also suggests that meditation-based interventions can promote wellbeing and reduce stress in healthy populations, and can lead to improvements in executive function and working memory (Cásedas, Pirruccio, Vadillo, & Lupiáñez, 2020; Chiesa & Serretti, 2009; Hoge et al., 2021; Whitfield et al., 2021). To understand the underlying mechanisms that link meditation practice to improved mental and physical health, researchers have begun to investigate the effects of meditation on biomarkers that are affected by psychological stress and that are associated with anxiety and depression. These biomarkers include cortisol (Bower & Irwin, 2016; O'Leary, O'Neill, & Dockray, 2016), cytokines and inflammatory gene expression (Black & Slavich, 2016; Bower & Irwin, 2016; Concetta, Teresa, Blerta, & Fabio, 2021), changes in telomere biology (Conklin, Crosswell, Saron, & Epel, 2019), and brain derived neurotrophic factor (BDNF; Gomutbutra, Yingchankul, Chattipakorn, Chattipakorn, & Srisurapanont, 2020). Here, we examine relationships between serum BDNF and immune cell telomere measures in the context of an intensive meditation retreat. We consider the interrelated functions of BDNF and telomere biology in the development and survival of cells throughout the body—highlighting neuroplasticity and immune cell function as two potential mechanisms by which meditation training might influence health.

[5] See Hoge et al., 2021 for the important caveat that, while meditation-based interventions consistently show salutatory effects, they generally do not outperform active controls.

Telomeres and Telomerase

Telomeres are DNA-protein complexes that flank the ends of eukaryotic chromosomes, protecting coding regions of DNA from instability and degradation. Because eukaryotic chromosomes are linear, they encounter the "end-replication problem" wherein approximately 50 nucleotides are lost from the ends of the chromosomes during each cycle of cell division (Lin & Epel, 2022; Zvereva, Shcherbakova, & Dontsova, 2011). Telomeres serve as a buffer to prevent the loss of genetic material during these divisions. As eukaryotic organisms age, their telomeres get incrementally shorter, rendering the gene-bearing portion of the chromosome more vulnerable to degradation. To compensate for this telomere shortening, some tissues express telomerase, a specialized ribonucleoprotein enzyme that is able to lengthen telomeres by replacing telomeric nucleotides (Greider & Blackburn, 1989). Accordingly, telomere length and telomerase activity are two of the factors that determine when cells enter replicative senescence or apoptosis—also known as programmed cell death (Masutomi et al., 2003).

Telomerase activity is most prevalent during embryonic development and in replicating tissues, including gametes, germ cells, and some adult stem cells (Zvereva et al., 2011). Most somatic tissues do not express telomerase and have a replicative capacity of about 50-70 cell divisions (Zvereva et al., 2011). Lymphocytes, a subtype of white blood cells, present an important exception to this rule, as multiple rounds of cell division are necessary for the adaptive immune system to mount a response when presented with a pathogen (Lin & Epel, 2022; Weng, 2008). While expressed intermittently and at relatively low levels, telomerase activity in lymphocytes is essential for proper immune function, and telomerase activity is highly regulated in the development and differentiation of T and B cells (Lin et al., 2010; Weng, 2008).

Moreover, average telomere length and telomerase activity in lymphocyte populations seem to correspond with their level of maturation and differentiation (Lin et al., 2010).

Immune cell telomere dysregulation has been implicated in a variety of diseases, and illustrates an important principle in biology: too much or too little can be problematic. On the one hand, excessively long telomeres are associated with some cancers and conditions of excess tissue growth (e.g., uterine fibroids and polyps). On the other hand, accelerated telomere shortening is linked to a number of age-related diseases, including Alzheimer's, type II diabetes, and cardiovascular diseases (Blackburn, 2005; D'Mello et al., 2015; de Punder, Heim, Wadhwa, & Entringer, 2019; Lin & Epel, 2022). Telomere shortening has also been linked to a number of psychiatric ailments, including anxiety, depression, and PTSD, and is thought to be one of the mechanisms by which psychological stress leads to disease (Epel & Prather, 2018; Lin & Epel, 2022). Accordingly, interventions that mitigate stress are assumed to promote more optimal telomere regulation, and may thus increase a participant's healthspan and overall wellbeing.

Brain Derived Neurotrophic Factor

BDNF is a well-characterized modulatory neurotrophin that mediates several neuroplastic processes, including neuronal differentiation, synaptogenesis, dendritic arborization, and synapse strengthening (Hempstead, 2014; Miranda, Morici, Zanoni, & Bekinschtein, 2019; Yang et al., 2014). It is one of several neurotrophins—a family of highly conserved growth factors expressed in the brain and peripheral tissues, which promote the survival, development, and function of neurons (Hempstead, 2014). As such, BDNF plays critical roles in the development and maintenance of brain circuits, neuronal morphology, and brain architecture.

In the brain, BDNF is most prominently expressed in the hippocampus, cortex, amygdala, striatum, and at lower levels in the hypothalamus (Brigadski & Leßmann, 2020). Hippocampal

BDNF, in particular, is thought to play a critical role in neuroplasticity, learning, and memory—with evidence implicating BDNF in spatial and contextual learning, learning of tool use, and episodic and recognition memory (Binder & Scharfman, 2004; Lubin, Roth, & Sweatt, 2008; Miranda et al., 2019). BDNF expression in the dentate gyrus—one of the only regions to demonstrate neurogenesis in the adult brain—is thought to be responsible for the remarkable structural plasticity of the hippocampus (McEwen & Gianaros, 2011). This is important, as neuroplasticity and the formation of new neurons are critical mechanisms by which organisms adapt to ever-changing environmental conditions (Calabrese et al., 2014), and the hippocampus is thought to be essential for facilitating emotional responses that account for situational context (Fox et al., 2014). Stress can suppress both neurogenesis and BDNF in the hippocampus, and hippocampal atrophy has been associated with chronic stress, anxiety, and depression (Fox et al., 2014; Goshen et al., 2008; Hölzel et al., 2011; Miranda et al., 2019; Sapolsky, 1996). Such impairments are assumed to reduce a person's ability to process information in new situations (McEwen & Gianaros, 2011), and can render individuals more susceptible to the systemic dysregulation characteristic of disease (Calabrese et al., 2014).

Given these crucial functions, it is not surprising that BDNF imbalances are associated with a range of neurodegenerative diseases and psychiatric disorders. For example, BDNF hyperactivity is associated with epilepsy and pain sensitivity (Dussán-Sarria et al., 2018; Iughetti et al., 2018), whereas lower levels of BDNF are associated with Alzheimer's, Parkinson’s, Huntington’s, schizophrenia, bipolar disorder, anxiety, major depression, and eating disorders (Binder & Scharfman, 2004; Brigadski & Leßmann, 2020). While best known for its roles in the brain, BDNF also plays critical roles elsewhere in the body. It is produced by immune cells (lymphocytes and microglia), endothelial cells, and smooth muscle cells, and is found in organs

such as the heart, lungs, liver, and kidneys. Accordingly, BDNF dysregulation is also found in a number of chronic health conditions, including coronary artery disease, diabetes mellitus, and inflammatory diseases (Brigadski & Leßmann, 2020).

Relationships between BDNF and Telomere Biology

Although the role of BDNF in the immune system is less understood, BDNF appears to play a role in immune cell function and mediates cross talk between immune cells and neurons (Schulte-Herbruggen, Braun, Rochlitzer, Jockers-Scherubl, & Hellweg, 2007). Like telomerase, BDNF is implicated in the regulation of immune cell development. For example, thymocytes, the hematopoietic progenitor cells that give rise to mature T cells, express the BDNF receptor trkB. Moreover, trkB expression is inversely related to the maturation and differentiation potential of thymocytes, suggesting that it is involved in their early development and survival (Maroder et al., 1996). Additionally, BDNF expression from activated T cells, B cells, and monocytes has been shown to support neuronal survival in vitro, suggesting that immune cells produce bioactive BDNF that serves a neuroprotective function (Kerschensteiner et al., 1999).

Similarly, telomerase appears to be integral for several of the same central nervous system functions that depend on BDNF, including cell proliferation, neuronal differentiation, neuronal survival, and neuritogenesis in neural stem and progenitor cells (Cheng et al., 2007; Liu, Nemes, & Zhou, 2018; Mattson & Klapper, 2001). Telomerase exists at high levels in the brain during embryonic development and declines gradually after birth (Greenberg, Allsopp, Chin, Morin, & DePinho, 1998; Martín-Rivera, Herrera, Albar, & Blasco, 1998; Wright, Piatyszek, Rainey, Byrd, & Shay, 1996). Likewise, TERT, the catalytic subunit of the telomerase holoenzyme, is highly expressed in neural stem cells and progenitor cells throughout the lifespan, but declines rapidly once these cells undergo differentiation (Caporaso, Lim, Alvarez-

Buylla, & Chao, 2003; Klapper, Shin, & Mattson, 2001; Kruk, Balajee, Rao, & Bohr, 1996; Miura et al., 2001). Importantly, these declines in telomerase following neuronal differentiation have been shown to increase cells' vulnerability to apoptosis (Armstrong et al., 2005; Fu, Begley, Killen, & Mattson, 1999; Fu et al., 2000). Additionally, rodent models have been used to demonstrate that telomerase mediates the neuroprotective effects of BDNF by inhibiting apoptosis in the context of spinal cord injury and hippocampal neuron development (Fu, Lu, & Mattson, 2002; Mattson & Klapper, 2001; Niu & Yip, 2011). Although the mechanisms remain largely unknown, these findings suggest that BDNF and telomerase play a coordinated role in promoting trophic effects in the central nervous system.

Interestingly, *Tert* expression may also play a role in mood and memory-related functions of the hippocampus. Using *Tert-/-* knockout mice, Zhou et al. demonstrated that the absence of hippocampal *Tert* results in depressive symptoms (2016), and that spatial memory formation is dependent on *Tert* expression in the dentate gyrus of the hippocampus (2017). There is also evidence that *Tert* expression can upregulate BDNF and other neurotrophic factors in the hippocampus (Baruch-Eliyahu, Rud, Braiman, & Priel, 2019). Interestingly, individuals with depression have been shown to express lower BDNF (Huang, Larsen, Ried-Larsen, Møller, & Andersen, 2014; Sen, Duman, & Sanacora, 2008), whereas antidepressants have been shown to upregulate BDNF expression in animal models and to normalize BDNF blood levels in people with depression (reviewed in Binder & Scharfman, 2004; Cattaneo, Cattane, Begni, Pariante, & Riva, 2016). These findings support the notion that both BDNF and telomere biology are involved in the mechanistic pathways associated with depression.

Chronic stress is implicated in the etiology of many of the degenerative and psychiatric disorders associated with BDNF and telomere dysregulation, including depression. Stress tends

to accelerate aging, and declines in BDNF and telomerase that occur with age are thought to play a role in the onset of age-related degenerative diseases, memory impairments, and cognitive decline (Lommatzsch et al., 2005; Miranda et al., 2019; Mizoguchi, Yao, Imamura, Hashimoto, & Monji, 2020; Sleiman et al., 2016; Ziegenhorn et al., 2007; though see De la Rosa et al., 2019 for contrary findings). In line with this notion, research indicates that several key mediators of the stress response influence both BDNF and telomere biology, including glucocorticoids, inflammatory cytokines, and oxidative stress (Lin & Epel, 2022). Moreover, many of the interventions believed to mitigate chronic stress, including exercise and social interaction, appear to improve BDNF and telomere regulation (Dinoff et al., 2016; Lin & Epel, 2022; Miranda et al., 2019; Szuhany, Bugatti, & Otto, 2015; Tarassova, Ekblom, Moberg, Lövdén, & Nilsson, 2020). Learning-associated cognitive training has also been shown to strengthen the BDNF pathway in rats, which may counteract age-related declines (Silhol, Arancibia, Maurice, & Tapia-Arancibia, 2007, reviewed in Miranda et al., 2019). Given their interrelated functions and responsiveness to the same types of stress-mitigating interventions, we investigated how BDNF and telomere biology are affected by the mental training and behavioral regulation involved in a month-long, residential meditation intervention.

Meditation, BDNF and Telomere Biology

Meditation practices have been developed across many wisdom traditions to facilitate personal and spiritual development (Dahl & Davidson, 2019; Gunaratana, 2011). The practices studied here, and that have received the most attention in the psychology literature, are drawn from Buddhist traditions and their secular adaptations. These practices are often used to cultivate a number of cognitive and socioemotional skills, including meta-awareness, the ability to direct one's attention volitionally, perspective-taking, and reappraisal (Dahl, Lutz, & Davidson, 2015;

Levit-Binnun, Arbel, & Dorjee, 2021; Lutz, Jha, Dunne, & Saron, 2015). The salubrious effects of meditation presumably stem, in part, from the cultivation of these cognitive and socioemotional skills, as well as from changes in world view and the development of an adaptive stance toward one's experience that can improve an individual's relationship with themselves and others. Theoretically, these skills provide strategies for managing anxiety and can help practitioners to supplant maladaptive, but habitual, stress responses with more adaptive alternatives (Conklin et al., 2019; Dahl et al., 2015; Fox et al., 2016; Lutz et al., 2015).

Given these qualities, meditation can be framed as a form of mental training, which—just like the learning of any other skill requiring self-regulation and focus—can promote structural and functional changes in the brain, including neuroplasticity (Davidson & Lutz, 2008; Davidson & McEwen, 2012). Given that BDNF is a central regulator of neuroplasticity, it is likely to be implicated in, or affected by, meditation training. A related possibility is that mind-body interventions, including meditation and yoga, facilitate neuroplasticity by reducing stress and stress-induced suppression of BDNF (Davidson & McEwen, 2012; Naveen et al., 2016).

Current evidence supports the hypothesis that forms of meditation training can increase BDNF and improve telomere regulation. A recent meta-analysis of 11 studies reported that mindfulness-based interventions were associated with increases in peripheral BDNF (Gomutbutra et al., 2020). Additionally, a total of 20 studies have examined telomere biology in relation to meditation (Conklin et al., 2019; Le Nguyen et al., 2019; Schutte & Malouff, 2014; Schutte, Malouff, & Keng, 2020). Of the 11 studies measuring telomerase activity, 9 found intervention-related increases or higher telomerase in the treatment group following meditation interventions, while two found no significant changes in telomerase activity. Meanwhile, only 3 studies, which involved interventions of relatively high intensity or duration, observed changes

in telomere length. While promising, these bodies of literature are subject to a great deal of heterogeneity in the interventions and populations studied, and have included very few control conditions.

The Present Study

Here we add to these literatures by examining BDNF and telomere biology in relation to one another in the context of a month-long Insight meditation retreat compared to a control group of similarly experienced meditators. We first examined whether the retreat intervention led to changes in serum BDNF. Finding no change in BDNF, we then examined relationships BDNF and measures of telomere biology. We found that Time 1 levels of serum BDNF predicted retreat-related changes in telomere length in our retreat group (reported in Chapter 1), despite there being no retreat-related change in BDNF.

METHODS

Participants

Retreat participants (n = 28; 14 female, 13 male, 1 transgender; mean age 51.9 years, range 27-69) were recruited from groups of meditators registered for one of two month-long meditation retreats held at Spirit Rock Meditation Center (SRMC) in Woodacre, CA. Control participants (n = 34; 23 female, 11 male; mean age 49.3 years, range 25-68) were recruited from the larger Spirit Rock community through flyers and presentations at weekly open house meditation classes. Control participants were required to have previously attended at least two retreats of 7 days or longer, and were selected to be similar to the retreat group in age, gender, and prior meditation experience.

Retreat Intervention

Insight meditation is a form of vipassana meditation rooted in the Theravadan Buddhist tradition. The retreats studied were month-long, silent, residential Insight meditation retreats offered annually at SRMC. Core practices taught during these retreats included mindfulness of breathing and redirecting one's focus to present moment-experience (Goldstein & Kornfield, 2001)[6]. Participants also received instruction in ancillary practices intended to cultivate aspirational qualities known as the four immeasurables: loving kindness, compassion, empathetic joy, and equanimity. Silent, residential retreats are designed to support continual practice throughout the day. The schedule for these retreats included ~11 hours of meditation practice (alternating between 30–45-minute intervals of sitting and walking practice), meals, and an hour of work meditation (e.g., kitchen or housekeeping duties).[7]

Blood Collection and Processing

Blood samples were collected by professional phlebotomists via antecubital venipuncture. Retreat participants (i.e., retreatants) were assessed onsite at SRMC and controls were assessed at the Anubhuti Retreat Center in Novato, CA—an isolated and quiet environment similar and in close proximity to SRMC. Retreatants' blood was collected between 5:00 and 6:00 AM on the morning following their first full day of retreat[8] and again 3 weeks later. Control

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[6] Recordings of the morning practice instructions are available on dharmaseed.org with codes QZSJ and PFZH.

[7] See Conklin et al. (2018) for more detail about the schedule and features of these particular retreats, and King et al. (2019) for information about the general structure and purpose of retreats.

[8] For some participants domestic or international travel was required to attend the retreat, which made blood collection prior to the onset of the retreat infeasible. Additionally, given the potential stress of travel logistics and departure from one's home for a full month, retreatants were given a full day to acclimate to the retreat environment. Therefore, the Time 1 measures used in this study were not a true pre-intervention baseline.

participants were also assessed at the beginning and end of a 3-week interval, during which time they maintained their normal daily routines. Control participants' blood was collected between 9:00 and 10:00 AM after completing a 40-minute meditation session[9]. Both groups were asked to fast for at least 8 hours prior to their blood draw.

At each time point, we collected whole blood in serum separator tubes (Becton Dickinson Vacutainers) for BDNF assays and in Cell Preparation Tubes (CPT, also from Becton Dickinson) to isolate peripheral blood mononuclear cells (PBMCs) for telomere length and telomerase activity assays (see Chapter 1 for further detail). Eight tubes, totaling 46 mL, were collected per participant. Samples were driven to a nearby field laboratory every 20 minutes to ensure that processing began as quickly as possible. Processed samples were then transported on dry ice to the UC Davis Center for Mind and Brain, where they were stored in a -80℃ freezer (Thermo Forma 88400) until they were delivered on dry ice to collaborating laboratories for analysis.

Serum

Following collection, blood was allowed to clot in serum separator tubes at room temperature for 30-45 minutes before being placed on ice. Samples were then mixed by inversion and centrifuged at 2750 rpm for 15 minutes at 4℃ to isolate serum, which was aliquoted into cryovials for future assays.

Brain-Derived Neurotrophic Factor

Serum BDNF was quantified in Dr. Synthia Mellon's lab at the University of California, San Francisco using the Human Free BDNF Quantikine ELISA kit (R&D Systems, Minneapolis,

[9] This meditation session was included to give control participants a chance to acclimate to the retreat center and to settle after driving from their homes.

MN, USA, Cat# DBD00). This sandwich enzyme-linked immunoassay kit bound free BDNF using a monoclonal antibody specific to human BDNF. A substrate was added to develop color proportionate to the amount of bound BDNF, and the optical density (OD) value of the color was measured using a spectrophotometer. The OD values were compared to a standard curve to determine the concentration of BDNF accounting for the serum dilution factor (which was typically 1:60, but in some cases, samples were run again at a 1:100 dilution). Each sample (derived from one participant at one time point) was run in triplicate on a single assay plate, with participants' Time 1 and Time 2 samples run on the same plate. The position of the Time 1 and 2 samples were alternated between participants so that any effects owing to the well positions were distributed across the Time 1 and Time 2 samples. Samples that resulted in a CV greater than 12% were run again in triplicate on one, and sometimes two, separate plates, thus resulting in 3 to 9 BDNF values per sample.

All BDNF values for each sample were mean-centered to the sample mean and compared to a pooled SD to identify outliers that may have resulted from technical error. Values that were more than 2.5 pooled SDs from the mean were removed from the dataset (out of a total of 252 Time 1 values, 6 values from 3 participants were marked for removal: out of a total of 245 Time 2 values, 4 values from 4 participants were marked for removal). With these outliers removed, the remaining values for each sample were averaged to compute a single BDNF estimate for each sample for use in further analyses.

Peripheral Blood Mononuclear Cells

Following blood collection, CPT tubes were kept at room temperature until centrifugation. At the field laboratory they were mixed by inversion and then centrifuged in a horizontal rotor for 30 minutes at 1600 rcf at 22℃. Mononuclear cells and platelets were

collected from just below the plasma layer and combined in a single 15 mL size conical centrifuge tube. Platelets were removed by two washes with Dulbecco's Phosphate Buffer Solution (DPBS). A subset of cells were dyed with 0.1% Trypan Blue and counted using a hemocytometer. PBMCs were then aliquoted and centrifuged for an additional 5 minutes at 7000 rpm at 4℃ before being prepared for telomerase activity and telomere length assays. For telomerase activity, PBMC cell pellets were suspended in 100µL TRAPeze® 1X CHAPS Lysis Buffer (Millipore S7705) and chilled on ice for 30 minutes. They were then centrifuged for 20 minutes at 13,000 rpm at 4℃, divided into 20µL aliquots, and stored in 0.5mL PCR tubes for future assays. For telomere length, no further processing of the PBMCs was required.

Telomerase Activity and Telomere Length

Telomerase activity and telomere length assays were conducted by Dr. Jue Lin in the Telomere Biology Core Lab at the University of California, San Francisco. For telomere length assessments, DNA was extracted from isolated PBMCs using QIAamp DNA Mini kits (QIAGEN, Cat# 51104) and stored at -80℃. Telomere length assay methodology was identical to that of Lin et al. (2010; adapted from Cawthon, 2002, 2009), with the exception that reaction mixes contained 1.78–12.6 ng of genomic DNA per 11 ml reaction. Telomerase activity was measured using the Gel-TRAP (Telomerase Repeat Amplification Protocol) method (Mender and Shay, 2015). The TRAPeze Telomerase Detection Kit (Millipore, Cat#S7700) was used to extract, amplify, and visualize the telomerase activity within a given sample. See Conklin et al. (2018) for additional details regarding the telomere length and telomerase assays.

Self-Report Measures

Physical activity was assessed with a subscale of the Lifestyle Profile II (Walker, Sechrist, & Pender, 1987). Respondents indicate the frequency with which they engage in listed

behaviors using a 4-point, Likert-type scale ranging from “Never” to “Routinely.” Item responses are averaged to produce sub-scale scores that range from 1-4.

Trait anxiety was assessed with 20 items from the State-Trait Anxiety Inventory (STAI-T; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). Participants indicated how they felt generally over the past month on a 4-point Likert-type scale that ranged from ‘‘Almost Never” to ‘‘Almost Always.” Item responses were summed to produce scores with a possible range of 20-80, with higher scores indicating greater anxiety.

Depression symptoms were assessed with the 20-item Center for Epidemiologic Studies Depression scale (CES-D; (Radloff, 1977). Participants indicated the frequency with which they experienced aspects of depression such as poor appetite, restless sleep, and feeling lonely over the last month using a 4-point Likert-type scale that ranged from ‘‘Rarely or none of the time (less than 1 day each week)” to ‘‘Most or all of the time (5–7 days each week).” Item responses were summed to produce scores with a possible range of 0-60, with higher scores indicating greater depression. Scores of 16 or greater identify individuals at risk for clinical depression.

Data Processing & Statistical Analysis

All analyses were conducted in R version 4.1.0 and R studio version 1.4.1717. For each variable, summary statistics were calculated (see Table 2.1) and distributions were examined to determine normality. Outliers in the biomarker data (i.e., BDNF, telomerase, and telomere length) were then detected and removed using the median absolute deviation (MAD) approach (Leys, Ley, Klein, Bernard, & Licata, 2013): Values that were more than 3 times the MAD from the median of the sample were marked for removal. This process resulted in the removal of 4 BDNF observations (3 Time 1, and 1 Time 2) and Time 1 and Time 2 telomere length observations for 1 participant. Telomerase and meditation experience variables were log

transformed. Because some participants had attended zero days of retreat in the past year, 1 was added to all values before the transformation.

Correlations were used to assess relationships between BDNF and demographic, mental health, prior retreats, and telomere variables at Time 1. Pearson's correlations were computed for tests involving normal and/or log transformed variables. Spearman's rank correlations were calculated for tests involving BMI and standardized scale variables that had a skew of more than .5 or less than -.5 (i.e., physical activity, anxiety, and depression). Spearman correlations are denoted in the results section with the subscript s.

To assess the effects of Group, Time, and the Group by Time interaction on serum BDNF levels, a mixed model ANOVA was computed in R using the rstatix package. Additional correlations were calculated to assess relationships between metrics of practice engagement and changes in BDNF, and between BDNF variables and changes in telomere biology. Linear regressions were then used to test whether BDNF at Time 1 predicted changes in telomere length when accounting for age, gender, and telomerase levels. Regressions were performed and assessed with the car, reghelper, and emmeans packages.

RESULTS

Correlations in the Full Sample at Time 1

Physical Predictors

Based on the literature reviewed above, we expected BDNF to correlate positively with physical activity and negatively with age. The literature also suggests there may be a relationship between BDNF and BMI, though these findings are more mixed (Alomari, Khabour, Alawneh, Alzoubi, & Maikano, 2017; Sandrini et al., 2018; Si, Zhang, Zhu, & Chen, 2021). To examine these relationships in our full sample, we tested correlations between BDNF and each of these

variables at Time 1, finding no significant correlations between BDNF and physical activity (r_s = -0.17, p = .215), age (r(57) = .09, p = .498), or BMI (r_s = .13, p = .326).

Mental Health Predictors

Based on the literature, we also expected a negative relationship between serum BDNF and depression (Cattaneo et al., 2016), and a possible relationship with anxiety—though the strength and directionality of this association is more mixed (Cattaneo et al., 2016; Suliman, Hemmings, & Seedat, 2013). Interestingly, we found a positive correlation between BDNF and anxiety (r_s = .31, p = .018) but not between BDNF and depression (r_s = .19, p = .17).

Prior Retreat Experience

Considering that BDNF may be influenced by meditation practice (Gomutbutra et al., 2020), we assessed the correlation between BDNF at Time 1 and the number of retreats a person had previously attended (r(55) = .04, p = .771). We also examined the relationship between BDNF and the number of days that a person had spent on retreat in the year immediately prior to participating in the study (r(53) = -0.07, p = .613), as this was the one metric of meditation practice where we observed a difference between groups. Neither association was significant.

Telomere Biology

Finally, to investigate relationships between BDNF and telomere biology, we examined associations between BDNF and telomerase and BDNF and telomere length in the full sample at Time 1. We found a negative correlation between BDNF and telomerase activity (r(55) = -0.35, p = .008), but no correlation between BDNF and telomere length (r(57) = -0.06, p = .666).

Retreat Effects

No Change in BDNF as a Function of Retreat

A mixed model ANOVA with BDNF as the dependent measure showed no significant group by time interaction ($F(1,52) = 2.59$, $p = .114$), suggesting there was no effect of retreat participation on BDNF levels (Figure 2.1). Likewise, there was no effect of Time ($F(1,52) = 0.1$, $p = .755$), but there was a main effect of group ($F(1,52) = 4.25$, $p = .044$), with the control group ($M = 27.45$ ng) having higher levels of BDNF than the retreat group ($M = 24.82$ ng) across timepoints, indicating that the groups may not have been well matched with respect to BDNF. Alternatively, this difference may be an early effect of the retreat that arose after one full day of practice.

Practice Measures

To assess whether people's level of engagement with meditation practice influenced how much their BDNF levels changed, we examined correlations between changes in BDNF and two metrics of meditation engagement. During their second assessment, we asked participants to rate how diligently they thought they had practiced in relation to their peers on a Likert-type scale ranging from 1-7 (Control $M = 4.0$, $SD = 1.49$, range: 1 to 7; Retreat $M = 2.79$, $SD = 1.41$, 1 to 6). We also asked them to estimate how many hours per week they had practiced during the study period (Control $M = 4.05$, $SD = 2.09$, range: 1 to 9; Retreat $M = 52.95$, $SD = 16.04$, range: 14 to 77). Interestingly, greater perceived diligence predicted greater BDNF increases in controls ($r_s = .45$, $p = .023$) but not retreatants ($r_s = .4$, $p = .055$). Similarly, more hours of practice predicted greater BDNF increases in controls ($r_s = .53$, $p = .007$) but not retreatants ($r_s = .32$, $p = .122$). Control participants who practiced ~5 or more hours a week had BDNF levels that were either maintained or increased over the study period.

Telomerase Activity

Given the functional relationships between BDNF and telomerase, we examined the correlations between these measures by group at each time point to investigate whether retreat participation strengthened the relationship between them. As described above, BDNF was negatively correlated with telomerase at Time 1 when the groups were combined. When examined separately, BDNF was negatively correlated with telomerase at Time 1 ($r(26) = -0.49$, $p = .008$) and Time 2 ($r(24) = -0.42$, $p = .031$) in the retreat group, but there was no correlation between BDNF and telomerase at either time point in controls (See Figure 2.2). We also examined the relationship between changes in BDNF and changes in telomerase activity from Time 1 to Time 2. Similarly, we found that changes in BDNF were negatively correlated with changes in telomerase across groups ($r(47) = -0.34$, $p = .017$), but when we assessed the groups separately, this relationship was significant in retreatants ($r(24) = -0.44$, $p = .024$) but not controls ($r(21) = -0.34$, $p = .112$).

Telomere Length

Next, we tested whether changes in BDNF were correlated with changes in telomere length. We found no significant relationship in retreatants ($r(24) = -0.22$, $p = .29$) or controls ($r(26) = -0.15$, $p = .461$). We then tested whether individual variability in basal BDNF levels predicted changes in telomere length. As depicted in Figure 2.3, retreat participants' BDNF levels at Time 1 predicted their retreat-related changes in telomere length ($r(24) = .61$, $p = .001$); no such relationship existed in controls ($r(26) = -0.2$, $p = .313$). Next, we conducted a linear regression to assess whether the relationship between BDNF at Time 1 and changes in telomere length in the retreat group held when accounting for age and gender. This model demonstrated that BDNF at Time 1 did indeed significantly predict changes in telomere length ($F(1,21) =$

16.54, $p < .001$), but age ($F(1,21) = 3.33$, $p = .082$) and gender ($F(1,21) = 0.03$, $p = .855$) did not. Parameter estimates indicated telomere length increased by 0.011 units (as measured in T/S ratios) for every nanogram increase in BDNF ($p < .001$, 95% CI (0.006, 0.016)). Finally, given the relationships observed between BDNF and telomerase and the functional relationship expected between telomerase and telomere length, we added telomerase activity at Time 1, and the interaction between BDNF and telomerase, to the model. We found no significant interaction between BDNF and telomerase at Time 1 and no main effect of telomerase.

DISCUSSION

Our primary aims in this study were to assess the effects of a month-long residential meditation retreat on participants' BDNF levels, and to investigate the relationships between BDNF and telomere biology in the context of intensive meditation training. We found that higher BDNF at the beginning of the retreat predicted greater increases in telomere length among retreat participants. Interestingly, we also observed negative relationships between BDNF and telomerase activity in the retreat group. Contrary to our predictions, we found no discernible group-level change in serum BDNF as a function of retreat. We did, however, find relationships between individual changes in BDNF and metrics of regular meditation practice in our control group, who were also experienced and practicing meditators.

BDNF and Telomere Biology in the Context of a Meditation Retreat

If we consider the canonical interpretations of these biomarkers, our results could suggest that BDNF levels—as a metric of participants' neuroplastic capacity—predict improvements in telomere biology consistent with greater longevity and lower disease risk as a result of participating in this residential retreat. In addition to this exciting possibility, there are a number of alternative explanations worth considering.

Given the existence of tissue-specific BDNF functions across the brain and in the periphery, we must also consider the functions of BDNF in the blood, and more specifically in serum. Like most human studies, we measured peripheral BDNF as a proxy for BDNF levels in the brain (Klein et al., 2011). However, BDNF levels measured in serum are also thought to result, in part, from the degranulation of platelets during coagulation (Cattaneo et al., 2016). Platelets are the primary site of BDNF storage in the blood, and play an important role in the regulation of BDNF homeostasis, suggesting that we should consider the functional implications of BDNF in the blood as well as the brain.

There are also at least two potential explanations for the changes observed in telomere length. One possibility is that we are observing true lengthening, which would suggest that telomerase, or some other mechanism of action, has elongated at least a subset of telomeres in these cells. In this case, it is tempting to conjecture that serum BDNF may be involved in a cell survival mechanism that lengthens telomeres. An alternative explanation for the telomere lengthening observed, often referred to as pseudo-lengthening (Epel, 2012), is that the composition of PBMCs measured at Time 1 differs from Time 2, such that the Time 2 samples contained a greater proportion of naive cells, or cell types with longer telomeres (such as B cells). In this case, it may be that serum BDNF represents the BDNF stores necessary for the development and survival of newly differentiated immune cells.

While we cannot distinguish between these two possibilities, our results do indicate that BDNF may be a useful biomarker in predicting who might experience health benefits as a result of intensive meditation training. It may also be useful to consider engaging in other BDNF promoting activities, like exercise, in preparation for retreat practice.

The negative correlations between BDNF and telomerase and individual changes in these measures in the retreat group are curious. Given that BDNF and telomerase seem to be involved in many of the same functions in the immune system and central nervous system, including cell development and survival, we would expect these measures to be positively related. Another possibility is that these biomarkers help to regulate one another and are thus anticorrelated during certain processes. The most we can conclude from our findings is that there is a relationship between BDNF and telomerase in our retreat group that exists after one full day of retreat and remains three weeks into training. This finding warrants further investigation to understand whether these biomarkers are involved in any mood or memory-related changes resulting from retreat participation.

Does Meditation Affect BDNF? Heterogeneous Findings and Future Directions

With respect to the question of whether or not meditation practice can modulate BDNF, we add both positive and null findings to a very mixed body of evidence, indicating that we must take stock of the variety of factors that might be contributing to these heterogeneous results. Factors to consider in the interpretation of our results, and as avenues for future research, include the intensity and styles of meditation training, measurement timing, the inclusion and assessment of exercise as an explicit intervention component, the role of proBDNF, the matrix in which BDNF is measured, and the various BDNF isoforms.

Advantages of studying a residential retreat include the high dose of meditation practice, immersion in a controlled environment with the goal of developing one's meditative faculties, and the ecological validity of studying a regularly offered retreat at a well-established retreat center. These features allow us to investigate developmental and process-oriented questions regarding what happens when experienced meditators dedicate time to developing and applying

their meditative skill set. Despite these advantages, coordinating with the retreat center and maintaining the integrity of the retreat necessarily limited our sample size and precluded randomization or a true pre-retreat assessment. These limitations make it difficult to distinguish baseline differences from early intervention effects[10].

When interpreting our findings, we must also consider the role of a month-long retreat in the developmental trajectory of an individuals' meditation training in concert with BDNF and telomere dynamics. Retreats provide an opportunity for concentrated reflection, personal insight, and integration that can lead to personal growth and healing. Moreover, all of our participants were committed enough to meditation practice that they had already undertaken at least two residential retreats, and our retreat group had committed to a month-long practice period of their own accord. With these motivational factors and prior experience at play, it is possible that our Time 1 data—which was collected after a full day of practice—reflect early effects of beginning such an endeavor in a physical environment that many consider a familiar refuge. As such, it may be that BDNF measured at Time 1 reflects early changes in the process leading to telomere change, rather than a basal individual difference.

Alternatively, if BDNF is associated with the acquisition of new skills, we might be more likely to see changes in practitioners attending their first retreat than in our participants, who were familiar with the retreat experience. Additionally, serum BDNF has been shown to increase in response to the Trier Social Stress Test, indicating that it can change in a matter of minutes to hours (Linz et al., 2019). In our study, we have two measurements taken 3 weeks apart, which

[10] For further discussion of the strengths and limitations of studying retreats see King et al., 2019.

unfortunately do not provide the temporal granularity necessary to thoroughly assess dynamic changes in BDNF across the course of the intervention.

It is also important to consider how tightly BDNF must be regulated given the range of critical functions it is involved in, and our controls offer thought-provoking data in this regard. In controls but not retreatants, we observed a positive correlation between hours of meditation practice and changes in BDNF. While largely speculative given the sample size, these data suggest that incorporating regular practice into one's daily life can promote BDNF. Retreat participants, on the other hand, engaged in significantly more practice than controls. If we consider the normal range of BDNF values, it may be that the association between BDNF and hours of meditation is not consistently linear across the range from 5 to 55 hours per week. Future studies with larger sample sizes and more frequent sampling should examine non-linear effects.

Another factor that merits consideration is the mean difference in practice diligence reported between the retreat and control groups. When asked to gauge how diligently they had practiced in relation to their peers, retreat practitioners reported practicing much less diligently than controls, on average, despite their having practiced for nearly 13 times as many hours. These differences suggest that our question evoked a very different meaning for individuals on retreat, who were surrounded by other meditators similarly committed to a rigorous schedule of practice, than for individuals practicing independently or with a group for shorter durations and at lower frequencies in their daily lives. The retreat schedule included approximately 11 hours of formal practice, or 77 hours per week. Given this implicit benchmark, it is reasonable then that retreatants averaging 55 hours per week could conclude they were practicing less diligently than their peers. For controls, on the other hand, reported diligence might be considered a measure of

how much they value or are able to prioritize their meditation practice amidst the demands of their daily lives. It then becomes interesting to consider whether this propensity for practice in controls is 1) supported by higher levels of BDNF, 2) contributes to higher levels of BDNF, or 3) if both outcomes are promoted by some third variable. Future research will be needed to distinguish these possibilities.

Another possible explanation for the discrepancies in the literature and the lack of a retreat-related change in BDNF observed in our study is the lack of an explicit physical activity component in our intervention[11]. Gomutbutra et al. (2020) concluded that across the 11 studies included in their meta-analysis, changes in BDNF observed in stationary mindfulness-based interventions (i.e., meditation) did not statistically differ from the increases observed in movement-based mindfulness interventions (e.g., Yoga and Tai Chi). However, it is interesting to note that all 5 of the studies investigating movement-based interventions found increases in BDNF, whereas only 2 out of the 6 studies investigating stationary mindfulness-based interventions found increases. Of the remaining 4 studies investigating non-movement interventions, 3 observed decreases in BDNF in the training group that were greater than or equal to those observed in the controls, and 1 found no changes in either group. Interestingly, the later findings are more consistent with the group difference we observed in BDNF at Time 1, with retreatants having lower BDNF. Further studies will be needed to directly compare mindfulness-based interventions with and without a physical activity component to delineate potential differences between these modalities.

[11] It is worth noting that, anecdotally, we know many practitioners do include exercise in their retreat routines, and spend time hiking in the local hills, but this was not a formal component of the schedule and was not measured.

It will also be advantageous for future research to differentiate between levels of proBDNF and mature BDNF. Like all neurotrophins, BDNF is initially synthesized as a precursor molecule (proBDNF), which is enzymatically cleaved to produce mature BDNF. Research suggests that BDNF regulates neuronal remodeling through the coordinated and opposing effects of pro- and mature BDNF (for a review, see Miranda et al., 2019). Mature BDNF binds to tropomyosin receptor kinase B (TrkB, also known as tyrosine kinase receptor B) to promote long-term synaptic potentiation (LTP) and dendritic complexity as well as synaptogenesis, differentiation, and neuronal survival (Jin, Sun, Yang, Cui, & Xu, 2019; Klein et al., 1991). Conversely, proBDNF binds to the p75 receptor (also known as nerve growth factor receptor or LNGFR) and can facilitate long-term synaptic depression (LTD) and apoptosis, which is an instrumental mechanism in neural pruning (Miranda et al., 2019; Yang et al., 2014). The ratio of these two proteins varies at different developmental stages, with the trophic functions of mature BDNF being more prevalent in adulthood (Yang et al., 2014, 2009).

In the present study we measured mature BDNF protein levels only. However, in the context of meditation training, the functions of both pro- and mature BDNF might be of interest, as a common goal of mindfulness training is to cultivate wholesome habits, while also noticing and intentionally diminishing unwholesome impulses. In fact, one of the proposed mechanisms by which meditation practice is thought to promote well-being is through extinction learning (Sevinc et al., 2020). This speculation is supported by findings in mice that suggest proBDNF/p75 activity is necessary for fear extinction (Ma et al., 2021), and that mindfulness training in humans is associated with connectivity changes in the hippocampus that are further associated with reductions in anxiety (Sevinc et al., 2020). Moreover, the apoptotic function of proBDNF is thought to be involved in the clearance or removal of damaged neurons in the

central nervous system (Kozlov, Grechko, Chegodaev, Wu, & Orekhov, 2020). If proBDNF serves a similar function in immune cells, then it is possible that the increases in average telomere length observed here might be the result of removing damaged cells with shortened telomeres.

Another potential contributor to the variation in BDNF and meditation findings is the substrate in which BDNF has been measured. In humans, the most feasible ways to measure BDNF in vivo are in plasma or serum. However, plasma and serum BDNF are thought to have potentially differing biological sources, biosynthesis mechanisms, and physiological roles (Tarassova et al., 2020). Recent evidence also suggests that plasma BDNF levels may be responsive to mental training, while serum levels respond to physical activity, but not mental training (Tarassova et al., 2020). On the other hand, animal and post-mortem studies indicate that BDNF in the brain is generally correlated with BDNF measured in serum, which might still make it a reasonable proxy for BDNF in the brain.

Moreover, there are 12 known isoforms of *bdnf*, which vary in their spatial and temporal expression, and are likely responsible for different functions. BDNF transcripts IV and IX are the predominant isoforms in blood leukocytes (Cattaneo et al., 2016), and are thus likely to be of particular importance in understanding the role of BDNF in the immune system. Ultimately, we are still very limited in our ability to assess the true range of tissue- and region-specific functions of BDNF in humans.

Conclusions

In sum, we found evidence that BDNF and telomere biology seem to play a coordinated role in the biological outcomes of sustained meditation practice. Given that our results are limited to peripheral measures, we cannot determine whether our measures reflect changes in the

central nervous system, the immune system, or both. While it will be challenging to directly assess these processes in the brain, it may be valuable to assess BDNF and telomere measures in PBMCs simultaneously to gain a clearer understanding of their roles in the immune system, the relationships between them, and how these relationships are influenced by meditation training.

As is the case with telomere biology, the regulation of BDNF expression and function is incredibly complex. Although it is infeasible to differentiate all of these regulatory pathways in vivo, especially in humans, future studies investigating the role of BDNF in meditation training will be most informative if they measure pro- and mature forms of BDNF and their receptors in plasma and in serum. It will also be informative to consider the full range of *bdnf* transcripts, and as demonstrated here, to consider how BDNF regulation relates to other critical systems. We should also be collecting several repeated measurements to tease apart the temporal dynamics of these mechanisms over the course of various meditation interventions. Finally, if the field of contemplative science is to push forward in understanding the role of such biomarkers in meditation training, we must formulate ways to conduct higher powered studies with ecologically valid interventions. This will require collaborations with multiple laboratories and retreat centers working together to conduct and replicate these studies.

TABLES

Table 2.1 *Descriptive Statistics*

	Control						Retreat					
	Time 1			Time 2			Time 1			Time 2		
	n	*M*	*SD*	*n*	*M*	*SD*	*n*	*M*	*SD*	*n*	*M*	*SD*
BDNF (ng)	31	26.79	4.62	29	28.11	6.34	28	24.94	4.93	26	24.28	4.67
Telomere Length (t/s ratio)	34	1.10	0.19	30	1.08	0.19	27	1.12	0.17	25	1.17	0.19
Telomerase (Ln)	32	2.16	0.60	27	1.99	0.62	28	1.87	0.70	26	1.71	0.49
BMI	34	24.58	3.63	30	24.93	3.83	28	23.64	3.98	26	23.18	3.69
Physical Activity	30	2.91	0.47	28	2.80	0.68	28	2.92	0.55	25	3.01	0.46
Anxiety	30	38.30	11.33	27	38.04	12.03	28	38.21	9.44	25	35.20	46.75
Depression	30	12.97	9.08	27	13.89	11.12	27	13.48	8.42	25	13.48	6.92

FIGURES

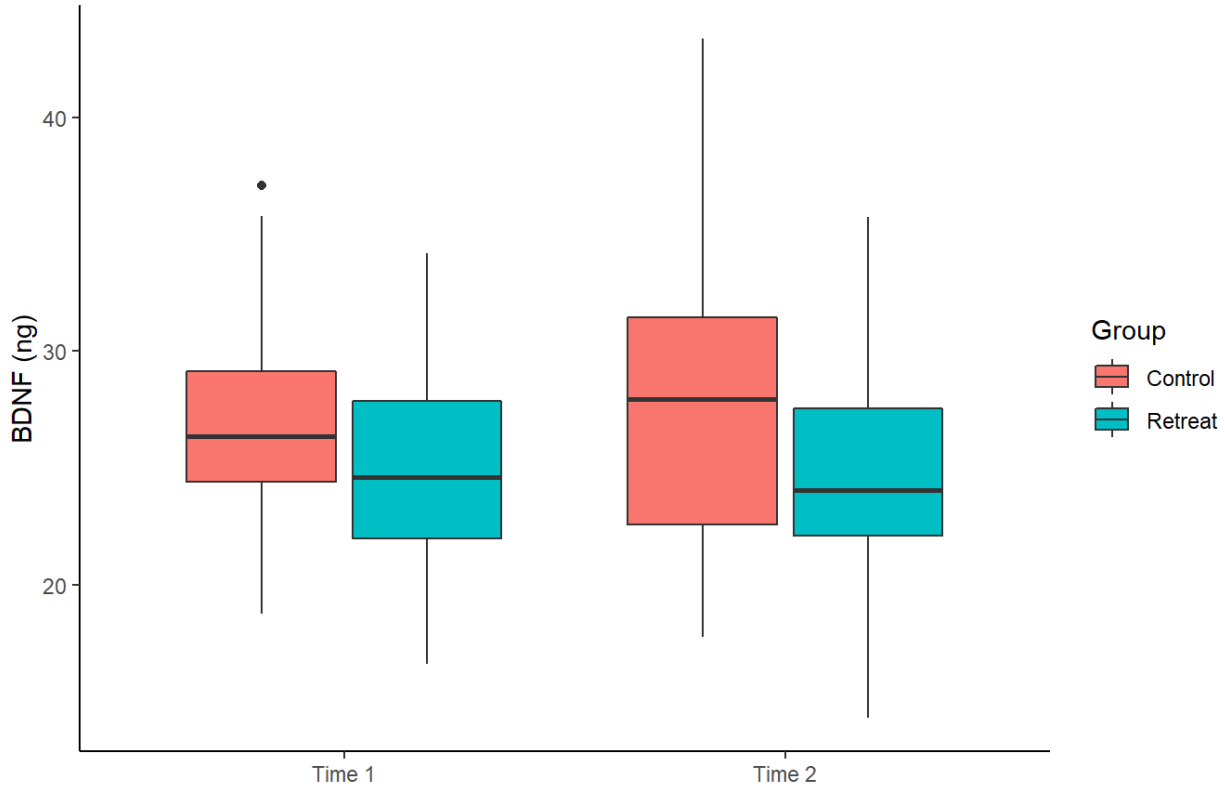

Figure 2.1. BDNF by Group and Time. Data plotted here represent participants with complete observations included in the mixed model ANOVA. Thus, the ns, means, and standard deviations differ slightly from those represented in Table 2.1, and are as follows: Control Time 1: n = 28, M = 27.09, SD = 4.59; Control Time 2: n = 28, M = 27.82, SD = 6.24; Retreat Time 1: n = 26, M = 25.36, SD = 4.76; Retreat Time 2: n = 26, M = 24.28, SD = 4.67.

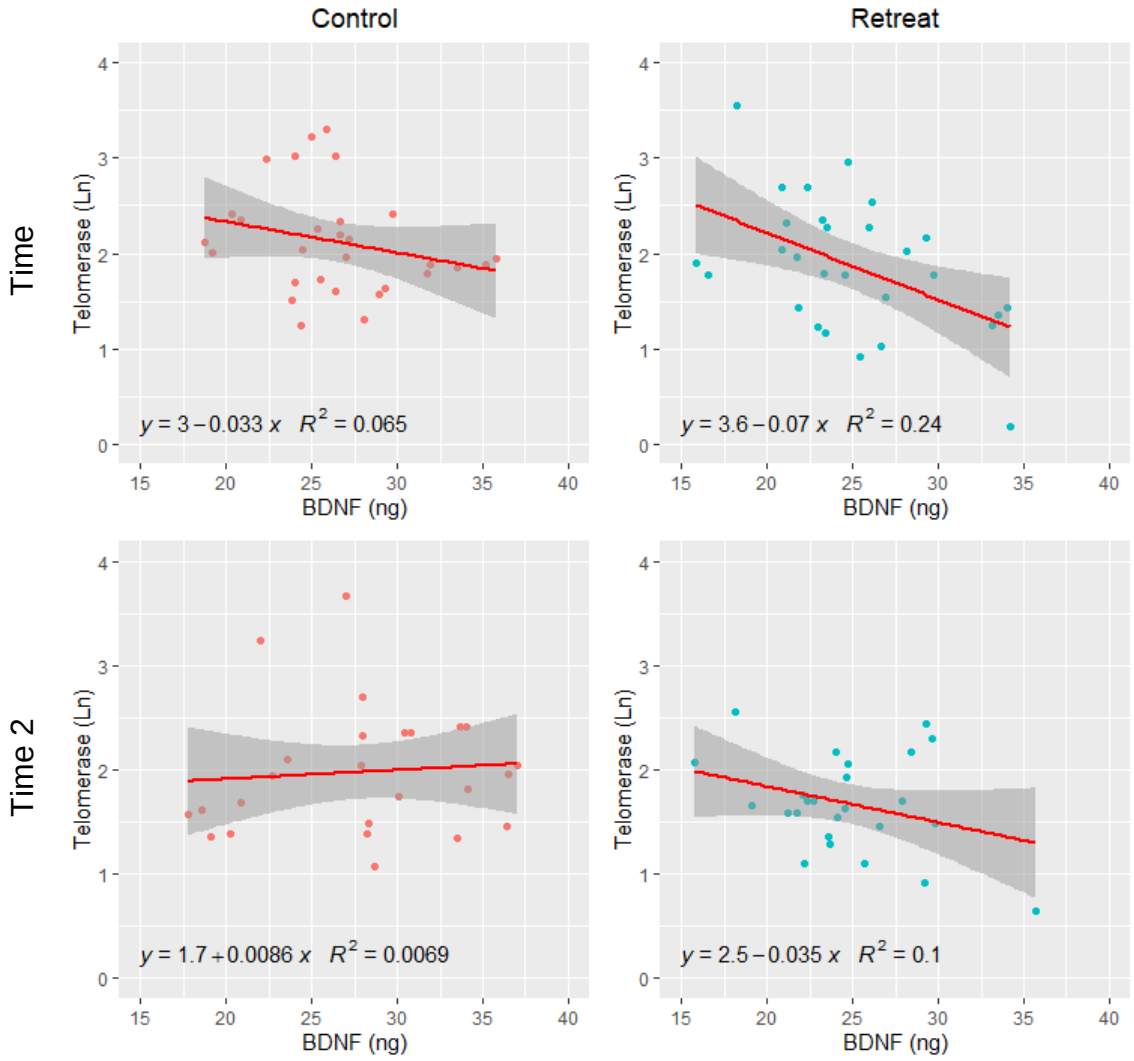

Figure 2.2. Correlations between BDNF and Telomerase across groups and time. As shown on the left, there was no correlation between BDNF and telomerase in the control group at Time 1 ($r(27) = -0.26$, $p = .18$) or Time 2 ($r(24) = -0.001$, $p = .996$). Shown on the right, are significant negative correlations between BDNF and telomerase at Time 1 ($r(26) = -0.49$, $p = .008$) and Time 2 ($r(24) = -0.42$, $p = .031$). The magnitude of these retreat group correlations are similar across time points, but R^2 values indicate that much more of the variance is explained by this relationship at Time 1 than Time 2.

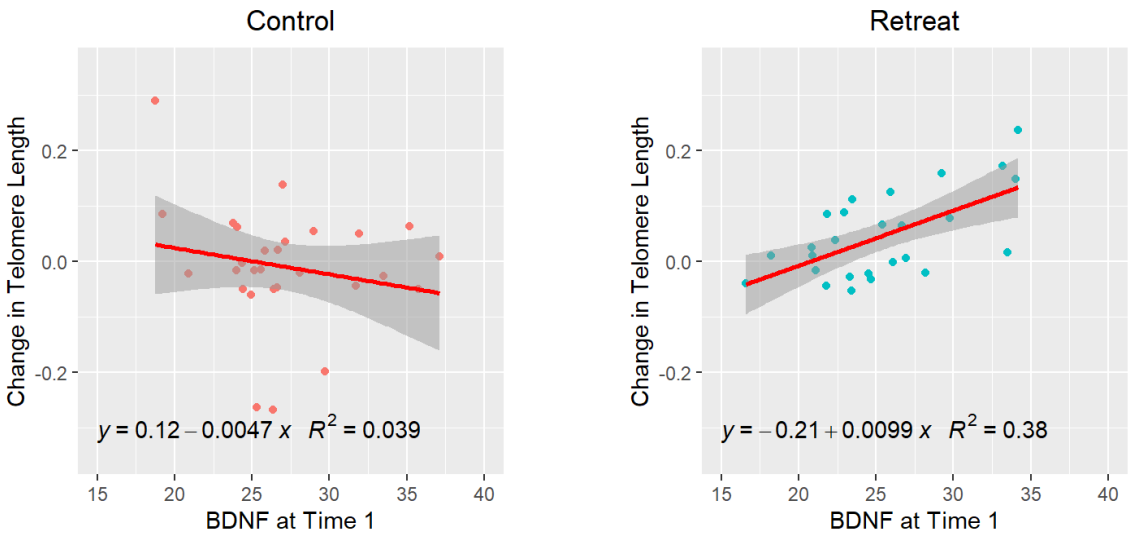

Figure 2.3. Time 1 BDNF predicts retreat-related changes in telomere length. As shown on the left, there was no correlation between BDNF at Time 1 and changes in telomere length in the control group ($r(26) = -0.2$, $p = .313$). However, BDNF at Time 1 did significantly predicted changes in telomere length in the retreat group ($r(24) = .61$, $p = .001$), as shown on the right.

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Chapter 3

Effects of an intensive meditation retreat on the expression of genes involved in inflammation and chromatin-modulation: Downregulation of the TNF-α pathway

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ABSTRACT

Background: Meditation retreats are characterized by intensive or concentrated periods of meditation practice, commonly undertaken in a residential retreat setting. Although research indicates that meditation training can positively influence physical and mental health outcomes, the biological consequences of this category of meditation intervention are relatively understudied. In this exploratory study, we examined the influence of a month-long, silent meditation retreat on the expression of genes involved in epigenetic and immune processes. **Method:** We assessed gene expression changes in experienced meditators attending a month-long Insight meditation retreat (n = 28), as compared to a control group (n = 34) of experienced practitioners living their everyday lives. Blood samples were collected on day two of the retreat (Time 1) and again 3 weeks later (Time 2). Control participants were also assessed across a 3-week interval, during which they maintained their regular daily routines. **Results:** Retreat participants showed significant changes in the expression of several genes involved in chromatin modulation and inflammation. The most substantive finding was the downregulation of genes involved in the TNF-α-pathway in retreat participants, which was not observed in controls. **Conclusions:** These findings indicate that meditation retreat participation may influence some of the inflammatory mechanisms involved in the development of chronic diseases, suggesting that this style of psychosocial intervention may have therapeutic potential, particularly for experienced practitioners.

***Keywords*:** meditation; mindfulness; retreat; gene expression; cytokines; interleukins; epigenetics

INTRODUCTION

Decades of research have demonstrated bidirectional relationships between the immune system and psychological well-being, with important implications for health and longevity (Dantzer & Kelley, 2007; Segerstrom & Miller, 2004; Slavich, 2015). Accordingly, there is a growing body of research aimed at understanding whether targeted lifestyle interventions, including meditation training, can bolster immune function (Black, Christodoulou, & Cole, 2019; Black & Slavich, 2016; Buric, Farias, Jong, Mee, & Brazil, 2017; Davidson et al., 2003; Kaliman, 2019; Kaliman et al., 2014; Rosenkranz et al., 2013, 2016). Changes in immune-related gene expression represent one way in which meditation training might bring about changes in the immune system. Initial evidence suggests that pro-inflammatory genes, which are upregulated in response to social stressors and early life adversity (Cole et al., 2012; Slavich & Cole, 2013), may be downregulated by mind-body interventions (Antoni et al., 2012; Buric et al., 2017). For example, a subset of our authors previously demonstrated that proinflammatory genes and genes involved in the epigenetic regulation of inflammatory processes were downregulated after 1 day of intensive meditation practice (Kaliman et al., 2014). In the present study, we extend this work by examining changes in gene expression across 21 days of intensive meditation practice in a residential retreat context.

Psychosocial Stress and Immune Responses

Immune responses require the rapid recruitment and production of inflammatory cell types (i.e., inflammation) when a pathogen is detected. This expansion is then, ideally, followed by a similarly rapid dissolution of these cells and a return to homeostasis when the threat has passed (Locksley, Killeen, & Lenardo, 2001). While acute inflammation is an adaptive and

protective response to injury and infection, serious health problems can arise when inflammation becomes chronic (Buric et al., 2017; Slavich, 2015).

The extensive tissue remodeling that occurs during immune responses depends on cytokines, a class of signaling proteins that regulate the growth and activity of immune cells. Tumor necrosis factor-alpha (TNF-α) is a pleiotropic cytokine that interacts with the receptors TNFRSF1A and TNFRSF1B to elicit differential outcomes, including inflammation, apoptosis, and necroptosis (Kalliolias & Ivashkiv, 2015). TNF-α is well known for driving inflammation by activating the transcription factor nuclear factor-kappa beta (NF-κβ; Bhatt & Ghosh, 2014; Diermeier et al., 2014), and is implicated in several inflammatory conditions (e.g., Sashio et al., 2002). Interestingly, NF-κβ and other intracellular transcription factors, such as IFN, can in turn drive TNF-α expression. NF-κβ also helps to regulate the survival, activation, and differentiation of innate immune cells and inflammatory T cells (Liu, Zhang, Joo, & Sun, 2017).

TNF-α and NF-κβ have been linked to a variety of psychosocial stressors and psychiatric conditions (Slavich & Irwin, 2014). For example, TNF-α pathway genes have been implicated in neuroinflammation (Palin et al., 2009) and depression (Kappelmann, Lewis, Dantzer, Jones, & Khandaker, 2018), and animal models have demonstrated that chronic stress can induce NF-κβ signaling (Koo, Russo, Ferguson, Nestler, & Duman, 2010). This is consistent with human research indicating that elevated inflammatory cytokines in serum (as indexed by IL-6, TNF-α, C-reactive protein and resistin) are linked to greater lifetime exposure to traumatic psychological stressors (O'Donovan, Neylan, Metzler, & Cohen, 2012). In contrast to the NF-κβ upregulation found in response to stress paradigms, early contemplative research suggests that these same pathways can be downregulated by mind-body interventions, including meditation, mindfulness, yoga, Tai Chi, Qigong, and breath regulation (Black & Slavich, 2016; Buric et al., 2017).

Epigenetic Regulation of Gene Expression

One way that psychosocial processes influence the immune system is by modulating underlying gene expression (Black et al., 2019; Cole, 2009; Slavich & Cole, 2013). Like all cells, immune cells derive their structure and functional characteristics, such as movement, metabolism, and biochemical responses to external stimuli, from proteins (Cole, 2009). Genes are the sequences of DNA that are *transcribed* into mRNA before they are *translated* into proteins, and it is the mRNA transcripts that constitute 'gene expression'. Because changes in gene expression precede changes at the protein level, gene expression can be a useful tool for detecting early effects of psychosocial interventions, and for better understanding the mechanisms that give rise to health-related changes.

Gene expression is regulated in several ways. To conserve space within our cells, genomic DNA is stored in the form of chromatin—a structure wherein DNA is tightly coiled around a core of histone proteins and further scaffolded by non-histone proteins. The conformation of this chromatin determines which genes can be expressed by regulating which genes transcriptional machinery can access. A closed, or condensed conformation will suppress gene expression by blocking access to the DNA, whereas an open or loose conformation promotes gene expression. Chromatin conformation is loosened by the addition of acetyl groups to histone proteins. Gene expression can, therefore, be regulated by the coordinated actions of histone acetyl transferases (HATs) and histone deacetylases (HDACs), which add and remove acetyl groups, respectively. Histone conformation can also be modified by the addition or removal of phosphate groups (via kinases and phosphatases) and methyl groups (via methyltransferases and demethylases). Some methyltransferases can also suppress gene expression by methylating DNA directly.

HDACs play an important role in regulating inflammatory signaling pathways, and dysregulated HDAC activity can cause or aggravate various disease states (Akimova, Beier, Liu, Wang, & Hancock, 2012; Gatla et al., 2019; Shakespear, Halili, Irvine, Fairlie, & Sweet, 2011). For example, HDACs appear to regulate TNF-α in a cell-type specific manner (Gatla et al., 2019). Given their role in regulating cytokines and chemokines, HDACs and HDAC inhibitors have garnered considerable interest as potential treatment strategies for various cardiovascular and kidney diseases, cancers, viral infections, and autoimmune disorders (Gatla et al., 2019); however, their widespread effects make it difficult to target specific illnesses and to assess their effectiveness. Because these processes are not easy to target pharmacologically, finding psychosocial interventions that positively influence these systems may be particularly valuable.

Meditation and Social Signal Transduction

Meditation and mindfulness-based interventions are hypothesized to improve health outcomes, in part, by promoting adaptive responses to stress (Conklin, Crosswell, Saron, & Epel, 2019; Creswell & Lindsay, 2014). Theoretical models propose that certain styles of meditation may influence stress appraisals and subsequent physiological responses by strengthening attentional control, decreasing rumination, and increasing one's ability to inhibit maladaptive and habitual stress responses (Black et al., 2019; Conklin et al., 2019; Epel, Daubenmier, Moskowitz, Folkman, & Blackburn, 2009). Some of these meditation techniques are specifically used to cultivate positive mind states and prosocial qualities believed to counteract negative thinking (i.e., compassion, loving-kindness, empathetic-joy, and equanimity). Collectively, these skills and qualities may bolster the psychosocial resources needed to buffer deleterious consequences of stress, and may be particularly helpful for attenuating physiological responses to interpersonal stressors (Conklin et al., 2019).

The theory of *social signal transduction* (i.e., the social regulation of gene expression Cole, 2009) offers an explanatory framework for understanding the effects of meditation practice on inflammatory gene expression (Black et al., 2019). Social signal transduction extends the biological concept of signal transduction—whereby extracellular signals, such as neurotransmitters and hormones, elicit intracellular changes in gene expression—to include the perception of social information. With this framework in mind, Black and colleagues (2019), argue that mindfulness can mitigate inflammatory gene expression by altering the interpretation of potentially threatening social stimuli.

In support of this idea, initial evidence from a 1-day mindfulness intervention study showed that reductions in *HDAC2* and *RIPK2* were associated with improved cortisol recovery following a Trier Social Stress Test (Kaliman et al., 2014). These findings suggest that there may indeed be a relationship between socially threatening stimuli and the regulation of inflammatory gene expression that is dampened or modified by meditative practice. This same study also showed reductions in *COX2* and several *HDAC* genes after 1 day of intensive meditation practice, whereas meditation-naïve control participants who engaged in 1 day of leisure activities (i.e., resting, reading and watching documentaries) showed no such changes (Kaliman et al., 2014). In this same cohort, experienced meditators showed increased methylation in genes involved in histone deacetylase recruitment and other forms of chromatin remodeling (Chaix et al., 2020). Taken together, these results suggest that meditation practice might elicit anti-inflammatory effects by regulating inflammatory gene expression via histone acetylation, methylation, and other chromatin remodeling mechanisms.

The Present Study

In the present study, we investigated a month-long meditation retreat intervention to characterize the psychobiological changes that occur when experienced practitioners engage in high doses of practice in a retreat context. Retreats are used to develop meditative expertise through extended periods of concentrated practice and are thus typically held in a secluded natural environment, where practitioners are removed from the distractions of daily life (King, Conklin, Zanesco, & Saron, 2019; Norman & Pokorny, 2017). The retreat structure is designed to encourage practitioners to use each moment and every activity during retreat as an opportunity for continued mindfulness practice. During retreat, meditators often follow a rigorous schedule of formal practice under the guidance of experienced teachers, and with the social support of fellow practitioners. Meditators may also adopt the practice of *noble silence*, which entails temporarily refraining from speaking, communicating, or initiating eye contact with others, except during meetings with teachers or to navigate common spaces. This practice is intended to facilitate quietude and a deeper focus on one's inner experience. Together these conditions afford a unique opportunity for participants to observe their mental experience, to cultivate particular qualities of mind, and to experience meditative insights that may have synergistic effects on their overall well-being.

Here, we build on prior findings from this month-long retreat (Conklin et al., 2018) by exploring the molecular mechanisms underlying inflammation and epigenetic processes. We assessed changes in the expression of 33 genes involved in inflammatory signaling and the epigenetic-regulation of gene expression in experienced meditators after 3 weeks of an intensive meditation retreat or 3 weeks of everyday life.

The initial aim of this study was to compare the gene expression changes observed in experienced practitioners after one day of intensive practice (Kaliman et al., 2014) to those observed after 21 days of intensive practice. The genes *HDAC2*, *HDAC3*, *HDAC9*, *RIPK2*, *COX2* and *TNFα* were chosen for this purpose. For these genes, we expected to see directionally similar patterns to those observed in the original study (Kaliman et al., 2014).

Our secondary aim was to expand on these findings by examining changes in other genes involved in inflammatory and epigenetic processes. For this aim, we selected 27 additional candidate genes based on 1) existing literature indicating their mechanistic role in the immune system, NF-κB signaling, and chromatin modification; 2) a preliminary analysis using human chromatin modification enzymes and inflammation RT² Profiler™ PCR arrays (Qiagen); and 3) a database analysis of physical and functional protein-protein interactions (String). This list included several cytokines, chemoattractants, and regulators of chromatin conformation and gene expression (e.g., histone deacetylases, acetyltransferases, and methyltransferases). Although we did not have specific hypotheses for each of these genes, we did expect to see retreat-related reductions in pro-inflammatory genes along with changes in chromatin modulation and other epigenetic gene-regulatory mechanisms that might support the suppression of these genes.

METHODS

Data presented in this report were collected as part of a larger investigation of intensive meditation training (ClinicalTrials.gov #NCT03056105). The purpose of the broader study was to evaluate the effects of a month-long residential retreat program on psychological well-being and psychobiological outcomes from an ecologically-informed perspective. We refer readers to our prior report for additional details regarding the retreat environment, meditation training, and participant recruitment and demographics (See Chapter 1 Table 1.1 and Conklin et al., 2018).

Participants

To maximize ecological validity, we chose to evaluate a long-standing and annually offered retreat program at a well-established retreat center. Retreat participants (i.e., retreatants, $n = 28$) were recruited from a pool of individuals pre-enrolled in one of two month-long residential retreats held at Spirit Rock Meditation Center in Woodacre, CA, USA. Given the length of the intervention and the logistical considerations of coordinating with a large-scale retreat center running established retreat programs, it was infeasible to randomize participants to groups. Instead, to control for some of the lifestyle factors that might be expected to accompany long-term practice, comparison participants (i.e., controls, $n = 34$) were recruited from the local Spirit Rock community. This control group had similar levels of lifetime meditation experience and prior retreat attendance, and did not significantly differ from the retreat group in age, gender, BMI, education or income level (see Conklin et al., 2018). Applicants who self-reported major medical and autoimmune diseases expected to impact immune function were excluded from the blood collection procedure. Two retreat and two control participants withdrew from the study after the initial assessment. Two additional controls could not attend the second assessment due to scheduling conflicts. Thus, the final sample consisted of 28 retreatants and 34 controls at the first assessment, and 26 retreatants and 30 controls at the second assessment. All participants gave informed consent before taking part in the study and were compensated at a rate of approximately $20 per hour for their time. The study protocol was approved by the University of California, Davis Institutional Review Board.

Meditation Retreats

Each retreat was taught by a team of six experienced meditation teachers who were not involved in the study design or implementation. Retreat participants lived at Spirit Rock

Meditation Center for the duration of their retreat, and maintained noble silence while practicing formal meditation for up to 10 hrs/day. Meditation practices included sitting and walking variants of Insight meditation (Goldstein & Kornfield, 2001), during which practitioners were instructed to direct attention to their bodily sensations (and movements, when walking) and to observe their thoughts as transient phenomena without grasping or over-identification. Participants were also instructed in practices intended to support the four immeasurables: loving-kindness, compassion, empathetic joy, and equanimity. Together, these practices aim to cultivate beneficial qualities of mind and positive aspirations for oneself and others.

Blood Sampling Procedure

Fasting blood was collected via antecubital venipuncture. Retreatants gave blood at Spirit Rock between 5:30-6:00 am the morning following their first full day of retreat and again three weeks later (one week before the end of retreat).[12] Controls were assessed at the Anubhuti Retreat Center in Novato, CA in small cohorts of 4 to 11 people over the course of the following year. Controls were also assessed at the beginning and end of a 3-week interval, during which they maintained their regular routines. At both assessments, controls gave fasting blood between 9:00-10:00 am after completing a 40-minute meditation session.

Quantification of Gene Expression

Whole blood was collected in Vacutainer Cell Preparation Tubes (CPT, Becton Dickinson) and transported to a field laboratory where peripheral blood mononuclear cells were

[12] Because some retreat participants traveled cross-country or internationally to participate, it was infeasible to collect blood before the retreat began. Additionally, we elected to give participants a full day to acclimate to the retreat environment, given the potential stress of travel and of leaving one's home, work, and family for a full month. As such, the first assessment was not a true pre-intervention baseline. We also elected to take the second assessment one week before the close of the retreat, as there can be a qualitative shift in experience as participants prepare to return home, and begin to re-engage in verbal communication and greater social interaction.

isolated by density-gradient centrifugation within 30 min of collection. Cell pellets were conserved in RNA Later (Sigma St Louis, MO) at −80°C and stored at the UC Davis Center for Mind and Brain until they were shipped to the University of Barcelona for RNA extraction in P. K.'s laboratory. Samples were shipped in and insulated shipping container with ~55lb of dry ice; they were in transit for four days and arrived frozen with dry ice remaining.

Total RNA was extracted using the mirVana™ RNA Isolation Kit (Applied Biosystems). Yield, purity, and quality of RNA were determined spectrophotometrically (NanoDrop, USA) using Bioanalyzer 2100 (Agilent Technologies). The resultant RNA had 260/280 nm ratios above 1.9 and RNA Integrity Numbers (RIN) higher than 7.5.

Quantitative real time (q-RT) PCR was performed for each sample in duplicate using a Bio-Rad CFX384 real-time PCR system (Bio-Rad) with TaqMan FAM-labeled specific probes (Applied Biosystems). The probes used are listed in supplementary Table 3.1. A pre-amplification step (TaqMan PreAmp Master Mix; Applied Biosystems) was performed for genes exhibiting very low levels of expression (*PRMT5*).

Q-RT PCR data were analyzed with Bio-Rad CFX Manager (Bio-Rad) using the automatic Ct setting to assign baseline and threshold Ct values. The expression of each gene was normalized to the expression of the *TBP* reference gene, and then the relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). Gene expression data were natural log transformed to correct for skew prior to analysis.

Multivariate Statistical Analyses

First, we examined differences in the overall profile of all 33 genes. Gene expression dissimilarities were compared between groups and assessments using multivariate distance matrix regression (MDMR; (McArdle & Anderson, 2001; McArtor, Lubke, & Bergeman, 2017;

Zapala & Schork, 2012)) with the *MDMR* package in *R* (McArtor, 2018). MDMR is a person-centered regression method that allows for the estimation of statistical associations between multivariate outcomes and categorical or continuous predictors based on dissimilarities among sets of data (McArtor et al., 2017). MDMR is ideally suited for analyzing genes defined by their participation in a biochemical pathway or other *a priori* grouping (Zapala & Schork, 2006). Dissimilarity was quantified using Euclidean distance between sets of genes.

We also calculated δ effect size statistics to estimate how much each gene contributed to the multivariate effects. δ represents the decrease in pseudo-R^2 when an individual gene is removed from the MDMR model relative to the estimate of pseudo-R^2 from the full model (McArtor et al., 2017). Effect sizes were calculated at Time 1 and Time 2 separately based on the between-groups comparison.

Univariate Statistical Analyses

Next, we analyzed changes in individual gene expression via linear mixed-effect models. For each gene, models included Group, Time, and the Group by Time interaction as well as age and BMI as covariates. Our primary analyses of interest were the Group by Time interactions (i.e., differential changes between groups over time). We also considered main effects of Group (i.e., group differences across both time points) because prior work indicated that gene expression differences can emerge after 1 full day of meditation practice (Kaliman et al., 2014), and our baseline assessment occurred after 1 full day of retreat. Our analyses yielded 66 test statistics in total (2 tests × 33 genes). Resultant *p*-values were subjected to false discovery rate (FDR) corrections to control for Type I error. The expected proportion of false discoveries was set at .05. Observed (uncorrected) and corrected *p*-values are reported in Tables 3.2 and 3.3.

RESULTS

Multivariate Gene Analyses

Multivariate distance matrix regression (MDMR) of Euclidean distances between the expression profiles of all 33 genes demonstrated that groups significantly differed at Time 1 ($p <$ .001) and at Time 2 ($p <$ .001). Both retreat ($p <$ .001) and control ($p =$ .012) participants' profiles changed over time, but this was qualified by a significant Group by Time interaction suggesting that retreat participants changed differently than controls ($p =$ .003). Neither age ($p =$ .347) nor BMI ($p =$ .347) significantly predicted overall gene expression profiles.

Univariate Group Differences across Assessments

Next, we examined individual genes. We first tested the main effect of Group on each gene. Fourteen analyses remained significant after correction (Tables 3.2 and 3.3). The retreat group had lower expression of the immune-system-regulating genes *COX2*, *RIPK2*, *HIF1A*, *GSTP1, Il1β*, *IL8*, and *TNFRSF1A*, but higher expression of *CXCR2* and *FKBP5*. The retreat group also had higher expression of the epigenetic-regulating genes *DNMT3A*, *HDAC9*, *KAT6B*, *KMT2G*, and *KMT5B.* Importantly, group differences in *COX2*, *IL8*, *TNFRSF1A*, and *HDAC9* were qualified by significant Group by Time interactions as described below. Group effects that were not qualified by an interaction effect are shown in Figure 3.3 (i.e., *Il1β*, *RIPK2*, *HIF1A*, *GSTP1*, *CXCR2*, and *FKBP5* shown in Figure 3.3A; *DNMT3A*, *KMT2G*, *KMT5B*, and *KAT6B* shown in Figure 3.3B). We observed no significant effects of Group or Group by Time interactions in *BCL3*, *CCR3*, *CCR5*, *CCR7*, *CXCL10*, *GADD45G*, *IL6R*, *IL6ST*, *IFNγ*, *NOD2*, *NR3C1*, *RETN*, *AURKC*, *HDAC2*, *HDAC3*, or *KAT7*.

Univariate Group Differences Emerging Over Time

In our primary analyses, we found seven Group by Time interaction effects that remained significant after FDR correction (Figure 3.4). Differential changes in expression between group over time (i.e., interaction effects) were observed for five immune system genes (*COX2*, *IL8, TNFα*, *TNFRSF1A*, and *TNFRSF1B*; Table 3.2) and two genes involved in histone modification (*HDAC9* and *PRMT5*; Table 3.3). Among these significant interactions, *COX2, IL8, HDAC9 and PRMT5* showed group differences at Time 1 with differential trajectories across assessments. *TNF* genes, on the other hand, showed no group differences at Time 1, but displayed a consistent pattern of retreat-related downregulation from Time 1 to Time 2.

Decomposing these effects, the retreat group (M = -1.98, SE = 0.15) showed lower *COX2* expression than controls (M = -0.08, SE = 0.13) at Time 1, $t(108) = 9.65$, $p < .001$, and at Time 2, $t(110.6) = 5.15$, $p < .001$ (Retreat: M = -1.19, SE = 0.15; Control: M = -0.12, SE=0.14). However, retreatants showed an increase in expression over time, $t(59.1) = -4.5$, $p < .001$, that was not observed in controls, $t(60.1) = 0.25$, $p = .806$.

Similarly, the retreat group (M = -1.69, SE = 0.18) showed lower expression of *IL8* than controls (M = -0.06, SE = 0.16) at Time 1, $t(96.7) = 6.80$, $p < .001$, and Time 2, $t(101.5) = 3.285$, $p = .001$ (Retreat: M = -1.03, SE = 0.18; Control: M = -0.20, SE = 0.17). Like *COX2*, *IL8* expression increased in retreatants over time, $t(57.9) = -3.55$, $p = .001$, but not controls, $t(58.4) = 0.81$, $p = .419$.

In contrast to the patterns above, retreatants (M = 0.40, SE = 0.05) showed higher *HDAC9* expression than controls (M = 0.04, SE = 0.04) at Time 1, $t(103.1) = -5.78$, $p < .001$. At Time 2, there was no difference in *HDAC9* expression between the retreat (M = 0.23, SE = 0.05) and control (M = 0.18, SE = 0.04) groups, $t(106.8) = -0.81$, $p = .417$. This pattern resulted from a

decline in *HDAC9* expression across assessments in retreatants, $t(59.1) = 3.18$, $p = .002$, alongside an increase in controls, $t(59.9) = -2.91$, $p = .005$.

For *PRMT5*, retreatants ($M = -0.37$, $SE = 0.10$) showed significantly lower expression than controls ($M = 0.01$, $SE = 0.09$) at Time 1, $t(110.5) = 2.61$, $p = .01$. At Time 2, the opposite pattern emerged, with the retreat group having higher expression ($M = -0.01$, $SE = 0.11$) than controls ($M = -0.38$, $SE = 0.09$), $t(110.7) = -2.61$, $p = .01$, due to an increase across assessments in retreatants, $t(56.1) = -2.59$, $p = .012$, alongside a decrease in controls, $t(57.1) = 3.03$, $p = .004$.

Interestingly, we observed evidence of retreat-related declines in the expression of *TNFα*, and the *TNFα* receptor genes, *TNFRSF1A*, and *TNFRSF1B*, as demonstrated by the emergence of group differences at Time 2 that were not present at Time 1 (Figure 3.4).

There was no difference between the retreat ($M = -0.05$, $SE = 0.06$) and control ($M = -0.04$, $SE = 0.06$) groups in *TNFα* expression at Time 1, $t(99.2) = 0.11$, $p = .914$. However, retreatants decreased in *TNFα* expression over time, $t(59.5) = 4.43$, $p < .001$, resulting in lower *TNFα* expression in retreatants ($M = -0.34$, $SE = 0.06$) than controls ($M = -0.07$, $SE = 0.06$) at Time 2, $t(103.6) = 3.12$, $p = .002$. Controls showed no change over time, $t(60.1) = 0.5$, $p = .621$.

Likewise, the retreat ($M = 0.008$, $SE = 0.05$) and control ($M = 0.004$, $SE = 0.04$) groups did not differ in *TNFRSF1A* expression at Time 1, $t(105.3) = -0.05$, $p = .96$. However, retreatants decreased in *TNFRSF1A* expression across assessments, $t(51.7) = 5.10$, $p < .001$, resulting in lower expression in retreatants ($M = -0.29$, $SE = 0.05$) than controls ($M = 0.00$, $SE = 0.05$) at Time 2, $t(108.5) = 4.19$, $p < .001$. Controls showed no change over time, $t(52.7) = 0.09$, $p = .933$.

The same pattern was observed for *TNFRSF1B*, with no difference between the retreat ($M = 0.11$, $SE = 0.06$) and control ($M = 0.01$, $SE = 0.05$) groups at Time 1, $t(115.7) = -1.5$, $p = .138$, but a decline in retreatants, $t(57.8) = 4.48$, $p < .001$, that resulted in lower *TNFRSF1B* expression

in retreatants (M = -0.24, SE = 0.06) than controls (M = -0.01, SE = 0.06) at Time 2, $t(116.2)$ = 2.75, p = .007. Controls showed no change over time, $t(59.1)$ = 0.06, p = .955.

DISCUSSION

Residential retreats are an increasingly popular form of meditation training, yet relatively little is known about how such retreats alter the molecular mechanisms underpinning health. Here, we addressed this gap by examining differences that emerged over the course of a month-long meditation retreat in the expression of genes directly and indirectly involved in the regulation of inflammation. We report a consistent pattern of downregulation in the TNF-α pathway, as indicated by retreat-related reductions in *TNF-α, TNFRSF1A* and *TNFRSF1B*. We also observed differential patterns in several other genes indicative of lower inflammation and greater transcriptional repression in the retreat group.

Downregulation of the TNF-α pathway

The downregulation of the TNF-α pathway observed in the retreat group is indicative of a retreat-related reduction in inflammation. Given recent interest in developing anti-TNF therapies to treat depression (Kappelmann et al., 2018) and inflammatory disorders (Steeland, Libert, & Vandenbroucke, 2018), our findings suggest that the non-pharmacological modulation of the TNF-α pathway by meditation retreats may be a useful tool for the prevention and treatment of such conditions.

These findings are particularly interesting in light of prior findings from this study, which demonstrated retreat-related increases in bulk telomere length (Conklin et al., 2018) that were predicted by basal levels of brain derived neurotrophic factor (BDNF, Chapter 2). BDNF expression is necessary for neurogenesis, and TNF-α negatively regulates neurogenesis through its *TNFRSF1A* receptor (Calabrese et al., 2014). Thus, the reductions observed in *TNF-α* and

TNFRSF1A may suggest an increased capacity for neurogenesis in our retreat participants, despite no change in BDNF protein at the group level. Additionally, the reductions in *TNF-α* may reduce telomere degradation, consequently contributing to better telomere maintenance.

These reductions in *TNF-α* alongside increased telomere length in our retreat group are consistent with findings by O'Donovan and colleagues (2011), who showed that shorter telomeres were associated with greater inflammation (TNF-α and IL-6). Our results are also consistent with the finding that 1 day of meditation practice led to enriched methylation of genes involved in the TNF-α and NF-kB pathways (*TBKBP1* and *TNFSF13B*; Chaix et al., 2020). Epel et al. (2016) also compared experienced and novice meditators attending a week-long retreat to a vacationing control group residing at the same resort and found that TNF-α protein levels were maintained in experienced meditators, but increased in the novice and vacation control groups.

Other Indicators of Lower Inflammatory Burden in Retreat Participants

COX2, *IL8,* and *Il1β* were consistently lower in our retreat group, despite increases in *COX2* and *IL8* expression across time. Moreover, effect sizes suggest that these genes contributed most to the multivariate differences observed between groups. Although we cannot distinguish whether these group differences are the result of early intervention effects or pre-existing group differences, they are consistent with lower inflammatory burden in retreatants.

Interleukin-(IL-)1β is a proinflammatory cytokine that activates microglia, regulates the activity of growth factors, and can stimulate immune cells to produce other proinflammatory cytokines (Audet & Anisman, 2013; Calabrese et al., 2014). Both TNF-α and IL-1β are implicated in sickness behavior and depressive phenotypes induced by stress (Dantzer & Kelley, 2007; Ja & Duman, 2008; Palin et al., 2009). In fact, IL-1β meditates the stress-induced inhibition of neurogenesis in the hippocampus, which suggests that it likely interacts with BDNF

(Calabrese et al., 2014; Ja & Duman, 2008). Coupled with downregulation observed in the TNF-α pathway, lower *IL-1β* expression in our retreat group may be indicative of a possible link between retreat experience and lower inflammatory activity.

In the retreat group, consistently lower levels of *IL8* were coupled with higher levels of *CXCR2*, which codes for one of two IL-8 receptors (the other being CXCR1 or IL8RBP). Despite having lower levels of *IL8* across time, the retreat group showed increases in both *IL8* and *CXCR2*. IL-8, also known as CXCL8, is a chemoattractant involved in the migration and activation of neutrophils during acute inflammation (Harada et al., 1994). IL-8 has been implicated in chronic inflammatory pain states (Penna et al., 2007) and is upregulated in the anterior cingulate cortex in mice with persistent inflammatory pain (Cui et al., 2012). Interestingly, Creswell et al. (2012) found lower *IL8* and *CXCR1* expression in older adults who were lonely, compared to those who were not lonely—but they observed no change in *IL8* or its receptor in relation to an 8-week MBSR intervention. On the other hand, Black et al. (2013) found *IL-8* downregulation in PBMCs following an 8-week Kirtan Kriya Meditation intervention, which is more consistent with the group difference we observed.

RIPK2 and *HIF1A* were also lower in retreatants than controls, and showed similar, but non-significant increases in the retreat group across time. These results are indicative of differences in NF-κβ signaling, and the group differences in *COX2* and *RIPK2* after 1 day of retreat are consistent with the reductions observed by Kaliman et al. (2014) after 1 day of meditation practice. We also observed higher levels of *FKBP5* in our retreat group. This is consistent with hypomethylation of the *FKBP5* gene observed by Bishop et al. (2018) in individuals with PTSD who responded to an MBSR intervention compared with non-responders who showed greater methylation of *FKBP5*. Finally, we observed lower levels of *GSTP1-1* in

our retreat group across assessments. *GSTP1-1* belongs to a family of glutathione S-transferases, which are antioxidant enzymes that play an important role in detoxification. This finding may suggest increased antioxidant activity in retreat participants, though it is somewhat contradictory to findings by Sharma et al. (2008), who report higher *GSTP1-1* expression in regular practitioners of Sudarshan Kriya—a breathing exercise used to induce relaxation.

Expression of Epigenetic Modulators

With respect to epigenetic modulators, we found few effects that could be strongly tied to the retreat intervention. In contrast to Kaliman et al. (2014), who found a pattern of decline in *HDACs* 2, 3 and 9 across 1 day of meditation, we found no effects in *HDAC2* or 3, and an increase in *HDAC9* in the retreat group. The controls showed a decline in *HDAC9* such that the groups were significantly different at Time 2, despite there being no group difference at Time 1. Retreatants also showed higher expression of the methyltransferases *DNMT3A, KMT2G,* and *KMT5B*, and the acetyltransferase *KAT6B* across both time points.

DNMT3A interacts with HDACs (Fuks, Burgers, Godin, Kasai, & Kouzarides, 2001) and the repressive histone-methyltransferases, KMT2G and PRMT5, to silence gene transcription (Li et al., 2006; Tsutsui et al., 2013). In our data, the retreat group had higher *PRMT5* expression at Time 1 and showed a significant decline in expression by Time 2, in contrast to the controls who were lower at Time 1 and significantly increased by Time 2. Expression of *KMT2G* (also known as SETD1B) was higher in the retreat group at both time points, but declined similarly in both groups. Interestingly, KMT2G positively regulates the activation of genes downstream of NF-κβ (Wang et al., 2012). KMT5B is involved in the methylation of histone 4, and the loss of this methylation with age is associated with human cancers (Fraga, Agrelo, & Esteller, 2007; Fraga et al., 2005). KAT6B can promote and suppress transcription (Champagne et al., 1999) and is

involved in the epigenetic regulation of neurogenesis in the mammalian brain (Sun, Sun, Ming, & Song, 2011).

Collectively, this profile suggests greater transcriptional repression, which could contribute to lower inflammatory expression, in the retreat group. However, the logistical constraints of our study design make it impossible to determine the source of these group differences, and thus make it difficult to interpret the health-related implications of these findings. It is possible that these group differences reflect effects of meditation that were initiated during the first full day of retreat and maintained by subsequent retreat practice. However, it is also possible that they reflect pre-existing group differences, or seasonal or circadian fluctuations, as the control samples were collected at various times throughout the year and at a different time of day than retreatants. Future research involving randomization to short-term interventions could clarify these possibilities and the health-related implications of these effects.

Strengths and Limitations

One of the strongest methodological advantages of our study is the ability to examine the effects of a concentrated dose of practice in experienced practitioners (King et al., 2019). Most meditation research has focused on the effects of short-term interventions as they are easier to control and interpret. However, for many people, meditation practice can be thought of as a life-long endeavor to cultivate well-being and to mitigate suffering in their lives. Meditation practice can also be effortful or puzzling at first; yet, a practitioner's experience of, and relation to, meditation is bound to shift over time. It is, therefore, important to investigate how psychobiological processes differ between short- and long-term interventions, and for practitioners with varying degrees of prior experience. For example, Kaliman et al. (2014) found no change in *TNF-α* expression after 1 day of practice, but we find significant downregulation

after 21 days of practice, suggesting that modulation of the *TNF-α* pathway may require more intensive practice.

Another major advantage of our study is the use of targeted gene probes. Many of the initial studies examining meditation-related changes in gene expression have relied on genome-wide microarrays. These extensive arrays are valuable exploratory tools to begin to understand which pathways might be influenced by a particular intervention, but require targeted follow-ups using more reliable assays as we have done here.

Unfortunately, studying a well-established and regularly offered retreat also comes with necessary logistical and methodological limitations (King et al., 2019). For instance, given the length and intensity of the retreat intervention, it was infeasible to randomly assign participants to the retreat or control groups, or to devise a comparable sham intervention. Instead, we aimed to maximize the ecological validity of our participant sample by recruiting a comparison group with an existing interest in meditation, an ongoing practice, and prior retreat experience. This conceivably allows us to better generalize our results to a population of like-minded individuals as opposed to a population of non-practitioners (see Rosenkranz, Dunne, & Davidson, 2019). However, the lack of randomization and an active control condition preclude causal inference. While our results do indicate retreat-related changes in *TNF-α* and its receptors, true randomization would be necessary to determine whether the retreat caused these changes. We also cannot determine whether non-meditation features of the retreat (e.g., vegetarian diet, natural environment) contributed to the differences observed.

Our study also lacks a true baseline assessment, which inhibits our ability to distinguish true group differences from early intervention effects, as seen by Kaliman et al. (2014) after 1 day of practice. A further limitation of this study is our inability to control for potentially

confounding seasonal variables. In this case we were unable to assess the retreat and control participants simultaneously, or in a stratified manner. As gene expression can be responsive to environmental factors such as temperature and daylight hours (Dopico et al., 2015), it will be important for future studies to stratify assessments and to devise methods to account for these potentially confounding variables. Studying an established retreat also limited our sample size. Repeated studies investigating shorter retreat interventions will be important for overcoming these logistical constraints and bridging gaps in the existing literature.

Conclusions

In sum, the differential expression patterns we observed between our retreat group and the comparison group of similarly experienced meditators living their daily lives suggest that engaging in retreat practice may promote beneficial changes in gene expression indicative of lower inflammation and lower disease risk. The data presented here also suggest that meditation retreats may support the treatment and prevention of inflammatory disorders, and suggest that future studies of meditation should target the TNF-α pathway. These findings are notable, as chronic, low-grade inflammation is associated with many modern health problems. Given the limitations of our study, the effects described here should be considered preliminary and require replication. Nevertheless, our results set the foundation for future randomized and controlled studies to assess the potential for intensive meditation-based interventions to complement treatment of inflammatory conditions.

TABLES

Table 3.1 *Primers and TaqMan FAM-Labeled Specific Probes Used for Real-Time q-PCR Analysis*

Target	Reference (ABI)	Reference Sequence ID
AURKC	Hs00152930_m1	NM_003160.2, NM_001015879.1, NM_001015878.1
BCL3	Hs00180403_m1	NM_005178.4
CCR3	Hs00266213_s1	NM_001164680.1, NM_001837.3, NM_178328.1, NM_178329.2
CCR5	Hs99999149_s1	NM_000579.3, NM_001100168.1
CCR7	Hs01013469_m1	NM_001301716.1, NM_001301717.1, NM_001301718.1, NM_001838.3
COX2	Hs00153133_m1	NM_000963.3
CXCL10	Hs01124251_g1	NM_001565.3
CXCR2	Hs01891184_s1	NM_001168298.1, NM_001557.3
DNMT3A	Hs01027166_m1	NM_001320893.1, NM_022552.4, NM_153759.3, NM_175629.2
FKBP5	Hs01561006_m1	NM_004117.3, NM_001145777.1, NM_001145776.1, NM_001145775.2
GADD45G	Hs02566147_s1	NM_006705.3
GSTP1-1	Hs02512067_s1	NM_000852.3
HDAC2	Hs00231032_m1	NM_001527.3
HDAC3	Hs00187320_m1	NM_003883.3
HDAC9	Hs00206843_m1	NM_001204144.1, NM_001204145.1, NM_001204146.1, NM_001204147.1, NM_001204148.1, NM_014707.1, NM_058176.2, NM_178423.1, NM_178425.2
HIF1A	Hs00153153_m1	NM_181054.2, NM_001530.3, NM_001530.3
IL1β	Hs01555410_m1	NM_000576.2
IL6R	Hs01075666_m1	NM_181359.2, NM_001206866.1, NM_000565.3
IL6ST	Hs00174360_m1	NM_175767.2, NM_002184.3, NM_002184.3
IL8	Hs00174103_m1	NM_000584.3
INFγ	Hs00989291_m1	NM_000619.2
KAT6B	Hs00202463_m1	NM_012330.3, NM_001256469.1, NM_001256468.1
KAT7	Hs01561260_m1	NM_001199155.1, NM_001199156.1, NM_001199157.1, NM_001199158.1, NM_007067.4
NOD2	Hs00223394_m1	NM_022162.2, NM_001293557.1
NR3C1	Hs00353740_m1	NM_000176.2, NM_001018074.1, NM_001018075.1, NM_001018076.1, NM_001018077.1, NM_001020825.1, NM_001024094.1, NM_001204258.1, NM_001204259.1, NM_001204260.1, NM_001204261.1, NM_001204262.1, NM_001204263.1, NM_001204264.1, NM_001204265.1
PRMT5	Hs01047356_m1	NM_001039619.2, NM_001282953.1, NM_001282954.1, NM_001282955.1, NM_006109.4
RETN	Hs00220767_m1	NM_020415.3, NM_001193374.1
RIPK2	Hs01572686_m1	NM_003821.5
KMT2G	Hs01051025_m1	NM_001243491.1, NM_012432.3, NM_001145415.1
KMT5B	Hs00992344_m1	NM_001300907.1, NM_001300908.1, NM_001300909.1, NM_016028.4, NM_017635.4
TNFα	Hs00174128_m1	NM_000594.3
TNFRSF1A	Hs01042313_m1	NM_001065.3
TNFRSF1B	Hs00961749_m1	NM_001066.2

Table 3.2

Immune Responsive Genes: Mixed-Model ANOVAs with Observed and Corrected p Values

Outcome	Gene Name	Predictor	*F* Value	$p_{observed}$	$p_{corrected}$
BCL3	B-cell CLL/lymphoma 3	Age	0.33	0.566	
		BMI	0.02	0.885	
		Group	3.72	0.059	0.118
		Time	4.76	0.034	
		Group by Time	0.44	0.511	0.636
CCR3	C-C motif chemokine receptor 3	Age	1.03	0.314	
		BMI	0.84	0.362	
		Group	1.02	0.316	0.443
		Time	20.06	<.001	
		Group by Time	0.17	0.685	0.779
CCR5	C-C motif chemokine receptor 5	Age	0.02	0.878	
		BMI	0.07	0.795	
		Group	0.05	0.83	0.869
		Time	3.79	0.056	
		Group by Time	0.17	0.683	0.779
CCR7	C-C motif chemokine receptor 7	Age	1.59	0.211	
		BMI	2.48	0.12	
		Group	4.78	0.033	0.083
		Time	7.14	0.01	
		Group by Time	5.48	0.023	0.067
COX2 *	cyclo-oxygenase-2 or prostaglandin-endoperoxide synthase 2 (PTGS2)	Age	1.06	0.308	
		BMI	0.91	0.343	
		Group	82.45	<.001	**<.001**
		Time	9.88	0.003	
		Group by Time	12.05	0.001	**0.006**
CXCL10	C-X-C motif chemokine ligand 10 or Interferon gamma-induced protein 10 (IP-10)	Age	3.18	0.079	
		BMI	0.38	0.538	
		Group	1.16	0.286	0.412
		Time	3.43	0.069	
		Group by Time	1.75	0.191	0.301
CXCR2	C-X-C motif chemokine receptor 2	Age	0.23	0.632	
		BMI	0.03	0.862	
		Group	11.23	0.001	**0.008**
		Time	20.55	<.001	
		Group by Time	0.11	0.745	0.82
FKBP5	FKBP prolyl isomerase 5	Age	0.24	0.629	
		BMI	1.46	0.231	
		Group	9.13	0.004	**0.015**
		Time	0.03	0.865	
		Group by Time	2.53	0.118	0.205
GADD45G	growth arrest and DNA damage inducible gamma	Age	0.7	0.406	
		BMI	0.67	0.417	
		Group	3.99	0.05	0.111
		Time	19.52	<.001	
		Group by Time	0.12	0.733	0.819

GSTP1	glutathione S-transferase pi 1	Age	0.21	0.65	
		BMI	0.08	0.781	
		Group	6.62	0.013	**0.04**
		Time	5.44	0.023	
		Group by Time	0.22	0.64	0.768
HIF1A	hypoxia inducible factor 1 subunit alpha	Age	2.25	0.139	
		BMI	0.07	0.795	
		Group	10.86	0.002	**0.009**
		Time	7.66	0.008	
		Group by Time	4.86	0.032	0.083
IL1β	interleukin 1 beta	Age	0.22	0.639	
		BMI	2.22	0.141	
		Group	24.04	<.001	**<.001**
		Time	0.3	0.588	
		Group by Time	0	0.96	0.96
IL6R	interleukin 6 receptor	Age	0.14	0.712	
		BMI	0.01	0.906	
		Group	0.24	0.623	0.761
		Time	6.21	0.016	
		Group by Time	5.43	0.023	0.067
IL6ST	interleukin 6 signal transducer	Age	0.11	0.739	
		BMI	5.36	0.024	
		Group	1.98	0.164	0.271
		Time	2.12	0.151	
		Group by Time	2.15	0.148	0.251
IL8	interleukin 8 or C-X-C Motif Chemokine Ligand 8 (CXCL8)	Age	0.83	0.365	
		BMI	1.54	0.22	
		Group	34.15	<.001	**<.001**
		Time	4.22	0.044	
		Group by Time	9.94	0.003	**0.011**
IFNγ	interferon gamma	Age	5.68	0.02	
		BMI	0.18	0.674	
		Group	4.08	0.048	0.109
		Time	0.37	0.544	
		Group by Time	4.28	0.043	0.103
NOD2	nucleotide binding oligomerization domain containing 2	Age	1.12	0.294	
		BMI	0.52	0.476	
		Group	3.38	0.071	0.134
		Time	6.91	0.011	
		Group by Time	4.27	0.044	0.103
NR3C1	nuclear receptor subfamily 3 group C member 1	Age	4.6	0.037	
		BMI	0.79	0.378	
		Group	1.42	0.239	0.359
		Time	3.06	0.087	
		Group by Time	0.7	0.408	0.539
RETN	resistin	Age	0	0.947	
		BMI	0.01	0.932	
		Group	3.6	0.063	0.122
		Time	59.14	<.001	
		Group by Time	3.26	0.076	0.136

*RIPK2**	receptor interacting serine-threonine kinase 2	Age	0.39	0.536	
		BMI	0.13	0.716	
		Group	9.61	0.003	**0.012**
		Time	3.43	0.069	
		Group by Time	5.06	0.028	0.078
*TNFα**	tumor necrosis factor alpha	Age	6.79	0.011	
		BMI	4.02	0.049	
		Group	3.77	0.057	0.117
		Time	12.96	0.001	
		Group by Time	8.51	0.005	**0.018**
TNFRSF1A	TNF receptor superfamily member 1A	Age	0.27	0.604	
		BMI	0	0.959	
		Group	6.92	0.011	**0.037**
		Time	14.54	<.001	
		Group by Time	13.63	0.001	**0.004**
TNFRSF1B	TNF receptor superfamily member 1B	Age	0.15	0.704	
		BMI	0.02	0.903	
		Group	0.83	0.367	0.504
		Time	11.13	0.001	
		Group by Time	10.6	0.002	**0.01**

Note: Expression values were natural log transformed prior to analyses. We FDR corrected the *p* values testing Group and Group by Time effects with the proportion of false discoveries (Q) across both effects set to .05. Significant effects are in bold font. Genes with a priori hypotheses are marked with an asterisk (*).

Table 3.3

Epigenetic Regulatory Genes: Mixed-Model ANOVAs with Observed and Corrected p Values

Outcome	Gene Name	Predictor	*F* Value	$p_{observed}$	$p_{corrected}$
AURKC	aurora kinase C	Age	0.04	0.848	
		BMI	0.04	0.835	
		Group	0.18	0.676	0.779
		Time	0.54	0.465	
		Group by Time	1.58	0.213	0.327
*DNMT3A**	DNA methyltransferase 3 alpha	Age	14	<.001	
		BMI	0.15	0.695	
		Group	7.57	0.008	**0.027**
		Time	0.85	0.361	
		Group by Time	3.34	0.073	0.134
*HDAC2**	histone deacetylase 2	Age	1.7	0.197	
		BMI	1.63	0.206	
		Group	1.83	0.181	0.292
		Time	0.79	0.379	
		Group by Time	1.15	0.287	0.412
*HDAC3**	histone deacetylase 3	Age	0.28	0.599	
		BMI	0	0.951	
		Group	3.77	0.057	0.117
		Time	0.03	0.862	
		Group by Time	0.47	0.497	0.63
*HDAC9**	histone deacetylase 9	Age	0.18	0.677	
		BMI	1.17	0.283	
		Group	15.11	<.001	**0.002**
		Time	0.14	0.714	
		Group by Time	18.53	<.001	**0.001**
KAT6B	lysine acetyltransferase 6B	Age	0.32	0.576	
		BMI	0.12	0.732	
		Group	9.88	0.003	**0.011**
		Time	0.02	0.899	
		Group by Time	0.8	0.374	0.504
KAT7	lysine acetyltransferase 7	Age	0.08	0.772	
		BMI	0	0.968	
		Group	0.09	0.76	0.822
		Time	0.49	0.486	
		Group by Time	0.01	0.943	0.957
KMT2G	lysine-specific methyltransferase 2G or SET domain containing 1B (SETDB1)	Age	0.31	0.582	
		BMI	3.02	0.087	
		Group	23.13	<.001	**<.001**
		Time	1.17	0.284	
		Group by Time	0.48	0.49	0.63
KMT5B	lysine methyltransferase 5B or suppressor of variegation 4-20 homolog 1 (SUV420H1)	Age	0.01	0.915	
		BMI	1.6	0.21	
		Group	12.13	0.001	**0.006**
		Time	4.33	0.042	
		Group by Time	0.06	0.8	0.851
PRMT5	protein arginine methyltransferase 5	Age	0.06	0.813	
		BMI	0.04	0.843	
		Group	0.01	0.941	0.957
		Time	0.01	0.921	
		Group by Time	15.63	<.001	**0.002**

FIGURES

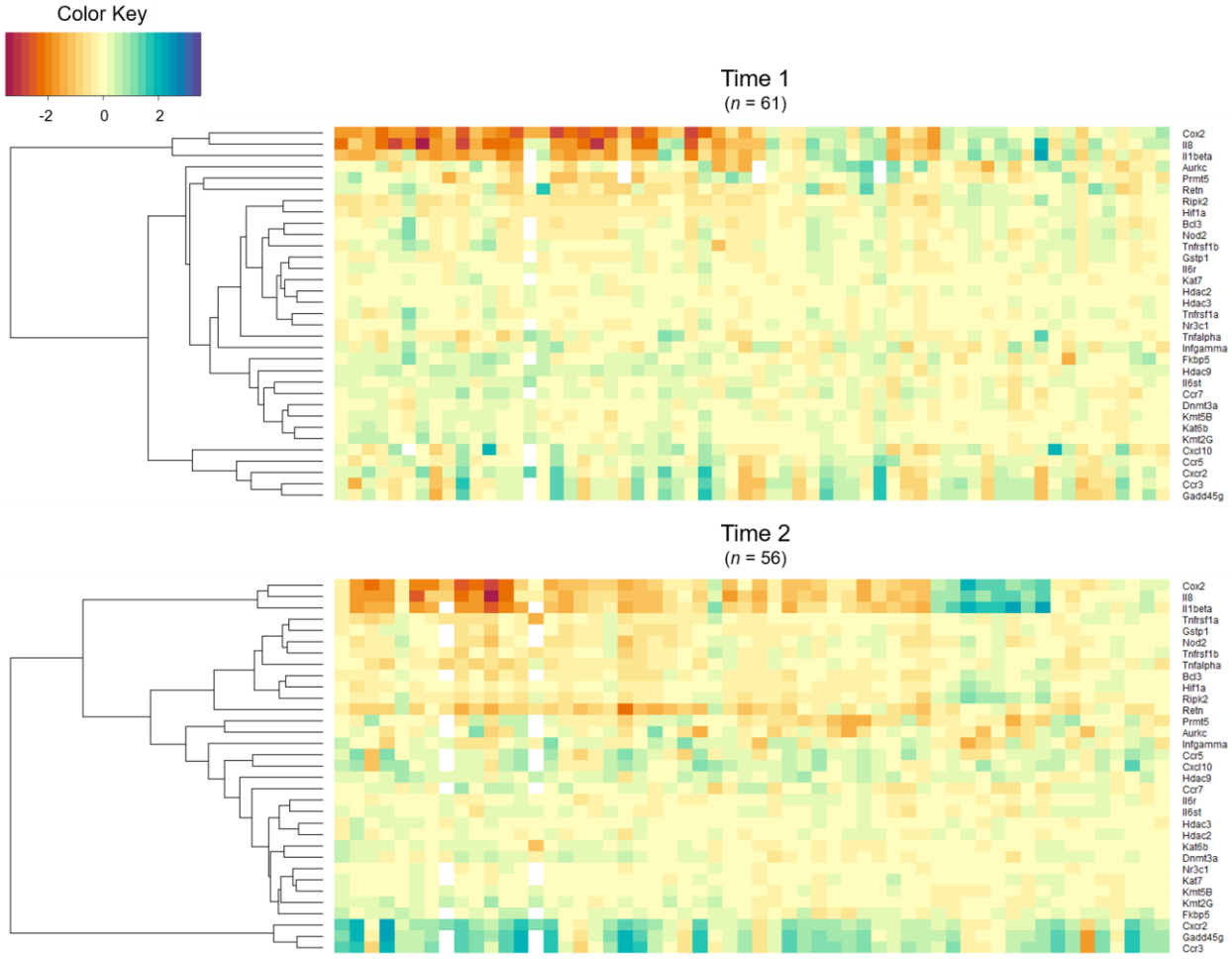

Figure 3.1. Dendrograms and heatmaps depicting relations between genes across participants at each time point. Retreat participants are plotted to the left and controls on the right. Red indicates lower gene expression and blue indicates greater expression. At both time points, we see that *COX2*, *IL8* and *IL1β* cluster together, showing similar patterns across participants. Interestingly, retreat participants show a fairly consistent pattern of downregulation at Time 1, with a subtle but visible increase in expression across participants at Time 2. These expression patterns are consistent with the group level changes visible in Figure 3.4. Controls, on the other hand, show much more variability and upregulation in these genes at Time 1, with three distinct categories of expression emerging at Time 2. This indicates that there is individual variability in these genes in the control group that is masked in the group mean. It is also of note that *CXCR2*, *GADD45G*, and *CCR3* cluster together at both time points, but show greater upregulation at Time 2. Moreover, *COX2*, *IL8* and *IL1β* expression appears to be inversely related to *CXCR2*, *GADD45G*, and *CCR3* expression, as these clusters show the greatest distance from one another in the dendrograms. Interestingly, *IL-8* is one of the genes with the lowest expression, while its receptor *CXCR2* is among those with the highest expression.

Figure 3.2. Gene expression heatmaps by Group and Time. Each row represents an individual participant; columns represent individual genes. Here, the most striking differences between groups lie in *COX2*, *IL8* and *IL1β*, which are notably lower in the retreat group at Time 1 as shown in the upper left panel. There is also a visible pattern of upregulation in *CCR3*, *CXCR2*, and *GADD45G* across groups at Time 2 shown in the panels on the right.

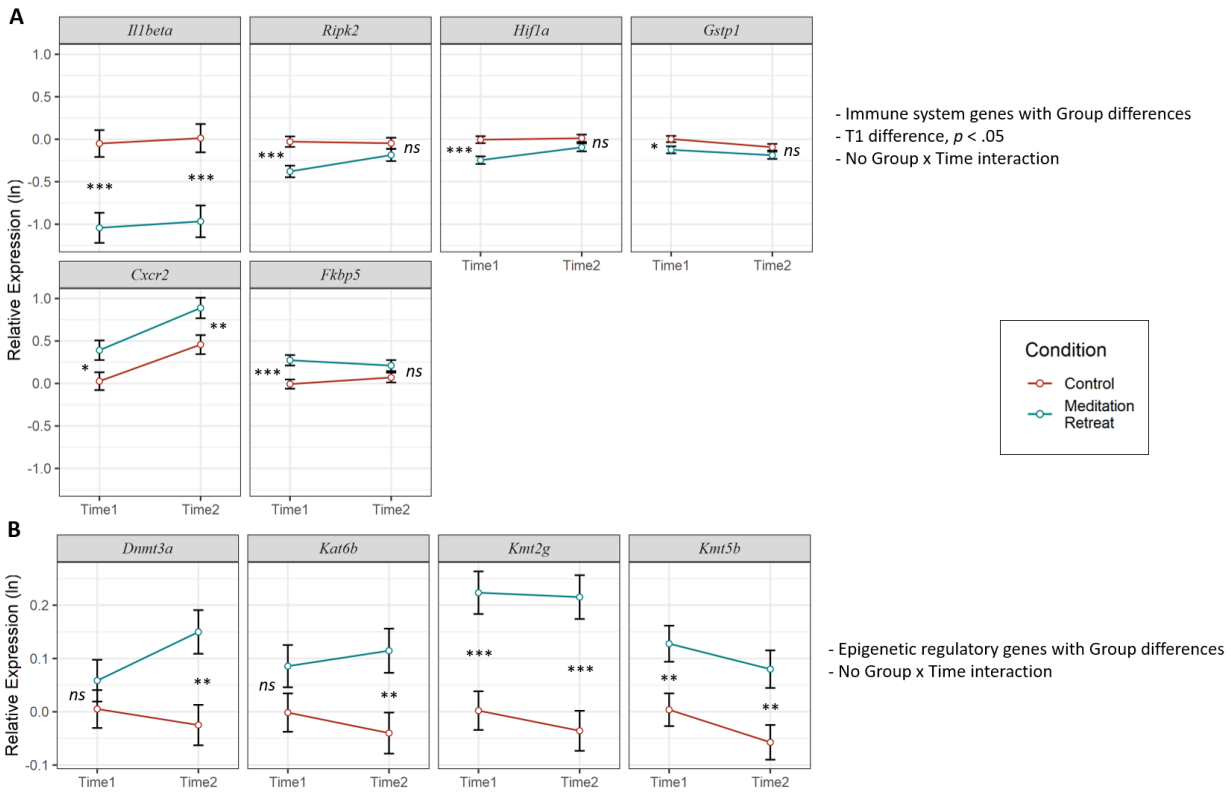

Figure 3.3. Significant Group that survived False Discovery Rate correction. Expression values were log transformed. Error bars represent standard errors of the mean. Group differences at Time 1 or Time 2 are indicated: *ns* = not significant, **p* < .05, ***p* < .01, ****p* <.001.

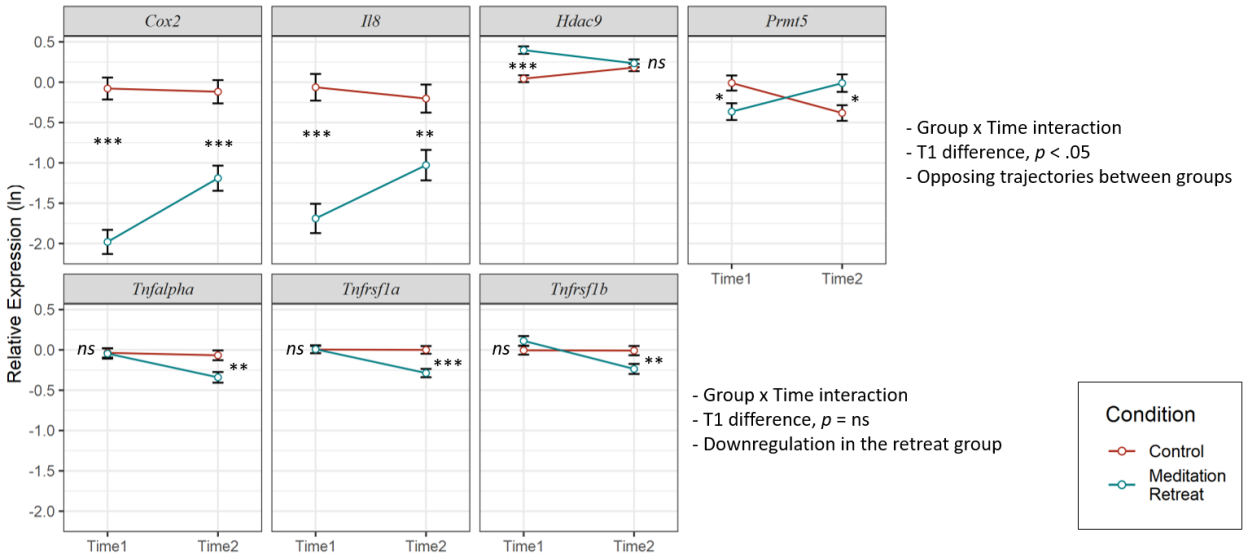

Figure 3.4. Significant Group × Time effects that survived False Discovery Rate correction. Gene expression values were log transformed. Error bars represent standard errors of the mean. Group differences at Time 1 or Time 2 are indicated: *ns* = not significant, **p* < .05, ***p* < .01, ****p* <.001.

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