FULL TEXT ARTICLE

Intensive meditation training, immune cell telomerase activity, and psychological mediators

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Summary

Background

Telomerase activity is a predictor of long-term cellular viability, which decreases with chronic psychological distress (Epel et al., 2004). Buddhist traditions claim that meditation decreases psychological distress and promotes well-being (e.g., Dalai Lama and Cutler, 2009). Therefore, we investigated the effects of a 3-month meditation retreat on telomerase activity and two major contributors to the experience of stress: Perceived Control (associated with decreased stress) and Neuroticism (associated with increased subjective distress). We used mediation models to test whether changes in Perceived Control and Neuroticism explained meditation retreat effects on telomerase activity. In addition, we investigated whether two qualities developed by meditative practice, increased Mindfulness and Purpose in Life, accounted for retreat-related changes in the two stress-related variables and in telomerase activity.

Methods

Retreat participants (n = 30) meditated for ~6 h daily for 3 months and were compared with a wait-list control group (n = 30) matched for age, sex, body mass index, and prior meditation experience. Retreat participants received instruction in concentrative meditation techniques and complementary practices used to cultivate benevolent states of mind (Wallace, 2006). Psychological measures were assessed pre- and post-retreat. Peripheral blood mononuclear cell samples were collected post-retreat for telomerase activity. Because there were clear, a priori hypotheses, 1-tailed significance criteria were used throughout.

Results

Telomerase activity was significantly greater in retreat participants than in controls at the end of the retreat ($p < 0.05$). Increases in Perceived Control, decreases in Neuroticism, and increases in both Mindfulness and Purpose in Life were greater in the retreat group ($p < 0.01$). Mediation analyses indicated that the effect of the retreat on telomerase was mediated by increased Perceived Control and decreased Neuroticism. In turn, changes in Perceived Control and Neuroticism were both partially mediated by increased Mindfulness and Purpose in Life. Additionally, increases in Purpose in Life directly mediated the telomerase group difference, whereas increases in Mindfulness did not.

Conclusions

This is the first study to link meditation and positive psychological change with telomerase activity. Although we did not measure baseline telomerase activity, the data suggest that increases in perceived control and decreases in negative affectivity contributed to an increase in telomerase activity, with implications for telomere length and immune cell longevity. Further, Purpose in Life is influenced by meditative practice and directly affects both perceived control and negative emotionality, affecting telomerase activity directly as well as indirectly.

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1 Introduction

1.1 Telomerase: linking stress with physical health

Although relations between psychological functioning and physical health have long been documented, mechanistic links are only beginning to be understood at the cellular level (e.g., Miller et al., 2009). Telomere length has recently been proposed as a useful 'psychobiomarker' linking stress and disease (Epel, 2009). Shortened telomere length and reduced telomerase (the cellular enzyme primarily responsible for telomere length and maintenance) predict a host of health risks and diseases (Blackburn, 2000; Serrano and Andres, 2004; Lin et al., 2009b), and new findings suggest they may be regulated in part by psychological stress, stress appraisals, and well-being (Epel et al., 2004, 2009a; Ornish et al., 2008; [3]). The literature on Buddhist traditions has long suggested that meditation can reduce psychological stress and enhance well-being (e.g., Dalai Lama and Cutler, 2009). In the present study, we investigated whether meditative practice is associated with immune cell telomerase activity and whether this association is at least partly explained by changes in two major contributors to the experience of stress: Perceived Control and Neuroticism (see Fig. 1 (fig0005)).

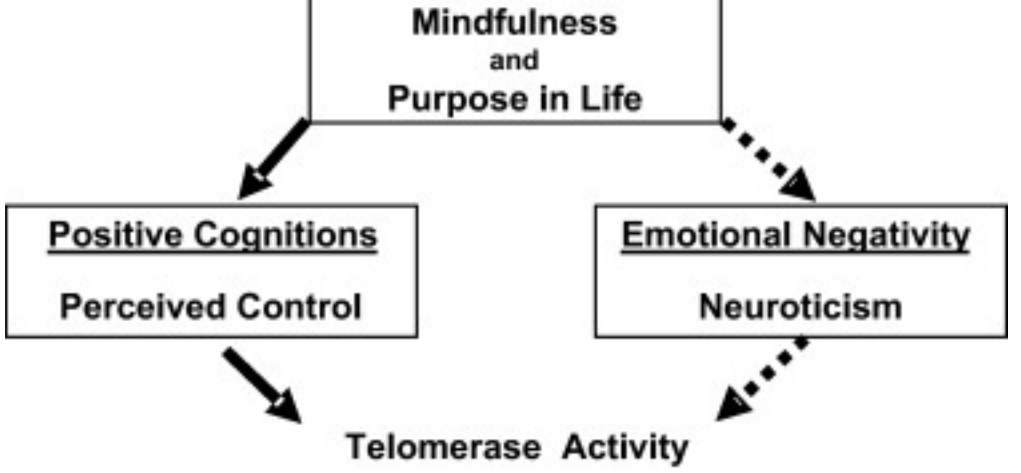

Figure 1

A schematic depiction of how meditation practice might influence telomerase activity by increasing perceived control and decreasing neuroticism (figure adapted from Epel et al., 2009a). Solid and dashed arrows depict positive and inverse relations, respectively.

Telomeres are protective DNA sequences at the ends of chromosomes that ensure genomic stability during cellular replication, but they shorten with each cell division and additionally shorten under conditions of oxidative stress unless counteracted by telomerase action (Blackburn, 1991). Below a critical telomere length, cell division can no longer occur and a cell is at a higher risk for entering a state of senescence, which may underlie tissue aging (Fossel, 2000; Chan and Blackburn, 2004). During human aging, as cells divide, telomere length decreases on average and hence is one indicator of a cell's biological age (Frenck and Blackburn, 1998), predicting physical health and longevity (e.g., Cawthon et al., 2003; Epel et al., 2009b; Njajou et al., 2009). Although cross-sectional studies show that telomere length decreases with age (on average), recent longitudinal studies indicate that in a significant fraction of people, peripheral blood mononuclear cell telomere length can increase over time (Epel et al., 2009b; Nordfjäll et al., 2009; Farzaneh-Far et al., 2010). This latter finding opens the door for determining potential malleable regulators of the rate of telomere length shortening. The rate of telomere shortening or lengthening depends on multiple factors including activity levels of the enzyme telomerase (Blackburn, 2000), which has the notable capacity to add DNA sequences back to telomeres, actively increasing their length and preserving healthy cell function (e.g., Kim et al., 2003).

Immune cell telomerase activity may play a role in mediating the relation between psychological stress and disease. Greater perceived stress, greater negative affect, and a number of stress-related cardiovascular risk factors (e.g., higher resting heart rate, elevated levels of stress hormones) are associated with lower telomerase activity (Epel et al. 2004). Moreover, individuals who respond to an acute stressor with suppressed vagal tone, which is an autonomic response that is inversely associated with negative affect and vulnerability to psychological stressors (Porges, 1995; Carney et al., 2001), also show lower telomerase activity, indicating a relation with psychological and physiological stress (Epel et al., 2006).

1.2 Perceived control and neuroticism as potential variables affecting telomerase activity during a meditation retreat

Given the relation between perceived stress and telomerase activity (Epel et al., 2004) and the literature on Buddhist traditions suggesting that meditation reduces psychological stress (Dalai Lama and Cutler, 2009), we reasoned that the specific psychological variables affecting immune cell telomerase activity during a meditation retreat might be the same ones that underlie individual differences in experienced stress. The great variation among individuals in experienced stress can be accounted for in part by other variables, including a feeling of inadequate control and the general propensity for negative affectivity, labeled neuroticism in personality studies. For example, a feeling of inadequate control has been associated in both classic and contemporary studies of animals and humans with greater psychological stress and less adaptive responses to stressful events (e.g., Seligman, 1972; Averill, 1973; Wallston et al., 1987; Vollmayr and Henn, 2001; Mausbach et al., 2006, 2008; Abelson et al., 2008; Karmilovich, 2009; [8]). Internal locus of control influences responses to stressful events by improving coping strategies (e.g., Krause and Stryker, 1984; Litt, 1988) and reducing anxiety (e.g., Chorpita and Barlow, 1998). Thus, perceived control is a key marker of stress resilience. Another contributor to stress is trait negative affectivity or neuroticism (chronically and characteristically feeling tense, anxious, moody, or insecure). High trait neuroticism typically amplifies stress responses in humans, particularly to acute stressors (Brody et al., 1996; Schneider, 2004; Shurgot and Knight, 2005), which leads to “greater stress vulnerability” (Suls, 2001).

Here, we explore the possibility that perceived control and neuroticism affect immune cell telomerase activity. Several physiological arousal processes may underlie relations between between perceived control and neuroticism with telomerase; they have been reviewed elsewhere (Epel et al., 2009a) and are not considered here.

1.3 Meditation practice as a potential regulator of perceived control and neuroticism

Increases in perceived control and decreases in neuroticism are plausible effects of meditative practice (e.g., Wallace and Shapiro, 2006). However, studies examining the effect of meditative practice on stress or well-being often raise methodological or interpretive questions. For example, self-selection into meditation training can result in a biased sample: Although lower neuroticism scores are reported by QiGong meditators who have practiced for a greater number of years (Leung and Singhal, 2004), meditation training is more likely to be discontinued by people with higher trait neuroticism (Delmonte and Kenny, 1985; Delmonte, 1988). Moreover, attributing outcomes to meditation per se can be tenuous, because it is often difficult to create an ideal control condition. One way to begin to handle such issues is to assess changes in measurable qualities that are specifically developed by meditative practice and determine whether they account, in a controlled, longitudinal study, for other positive outcomes.

One aspect of meditative practice is mindfulness, which is broadly defined in Buddhist traditions as the ability to maintain attention toward chosen meditative objects (Wallace, 2005) and also includes an emphasis on attending to beneficial thoughts (Gethin, 2001). Although Western concepts of mindfulness draw on these Buddhist views, they often diverge from this traditional understanding (Grossman, 2008; Wallace, 2008). Mindfulness is operationalized in contemporary psychological models as a multifaceted construct tapped, in part, by self-report measures of abilities to carefully observe and label internal or external experience in a non-reactive, non-judgmental manner (Baer et al., 2006). Studies have shown that this type of naturally occurring, dispositional mindfulness is inversely related to neuroticism (for a meta-analysis, see Giluk, 2009) and positively related to other forms of self-regulation and to positive emotions (e.g., Brown and Ryan, 2003). Mindfulness may be developed in practices that maintain attention on a meditative object (e.g., one's breath, awareness itself, actions and sensations of daily activity; see Wallace, 2006). Mindfulness-based training has been widely reported to improve psychological and physical well-being and to reduce stress (for reviews, see Grossman et al., 2004; Chambers et al., 2009; Rubia, 2009). However, aside from more recent exceptions (e.g., Baer et al., 2008; Carmody and Baer, 2008; Shapiro et al., 2008), most of these mindfulness training studies have considered mindfulness as an outcome measure along with other outcomes, rather than as a quality developed during a wider meditative practice that explains other positive results. Moreover, its effect on telomerase has never been assessed. Here, we test whether mindfulness mediates the effects of meditative practice on perceived control, neuroticism, and telomerase activity. We assign mindfulness this mediating role because it is an explicit target of meditation practice (Wallace, 2006), which is expected to bring about beneficial outcomes, such as better emotion regulation and greater emotional stability (e.g., Bishop et al., 2004; Brown and Ryan, 2003). Moreover, previous theoretical and empirical contributions to research on mindfulness have emphasized the causal role of mindfulness practices. They (e.g., Borders et al., 2010) have pointed out that such practices reduce negative rumination (which contributes to depression). In addition, a recent study assigned mindfulness a causal role in a model of positive reappraisal processes that contribute to adaptive coping (Garland et al., 2009 ; for review see Garland et al., 2010).

Mindfulness is only one quality cultivated by meditative practice and other qualities have only recently begun to be explored (e.g., Kraus and Sears, 2009; Sahdra et al., 2009). One understudied quality developed during meditative practice is a shift in intentions and priorities away from hedonic pleasure or superficial well-being (Nanamoli and Bodhi, 1995; Wallace and Shapiro, 2006), making one's deeper “purpose in life” (a measurable psychological construct; Ryff, 1989) clearer. Although meaning in life is a well-established correlate of other measures of psychological well-being (Ryff and Keyes, 1995; Klinger, 1998), it has never been examined as a benefit of meditation practice that might account for other positive outcomes. Here, we consider the possibility that enhanced purpose in life is one of the mediators of the relation between meditation practice and perceived control and negative affectivity and telomerase activity. We assign purpose in life this

mediating role based on a recent integrative model of the ways in which the sense of overarching meaning (extending beyond a particular situation) affects health outcomes via changes in specific psychological mediators (e.g., changes in appraisal or coping mechanisms that reduce negative affect) (for a review of this model, see Bower et al., 2008). The causal nature of the relation between meaning-finding and affect is supported by longitudinal studies, which suggest that the ability to create an overarching sense of meaning in the face of stressful events precedes a change in affect (for a meta-analysis, see Helgeson et al., 2006). With respect to meditation, Fredrickson et al. (2008) used causal path analyses and growth modeling to show that the amount of weekly time spent in 'loving-kindness' meditation predicted a cumulative, daily increase in positive emotion over a 2-month period. This increase contributed to an increase in life satisfaction and reduced depressive symptoms *via* the mediation of purpose in life and mindfulness.

1.4 The present study and hypotheses

Using a longitudinal wait-list controlled design, we determined whether participation in an intensive 3-month meditation retreat would result in increased mindfulness, an enhanced sense of purpose in life, greater perceived control, decreased neuroticism, and greater post-retreat immune cell telomerase activity. Mediation models were used to test two sets of predictions, based on the framework in Fig. 1 (fig0005) . First, we tested whether the meditation-related changes in post-retreat telomerase activity were mediated by any of the four measured psychological variables. Second, we tested whether retreat-related changes in indicators of positive cognitions and emotional negativity (i.e., increases in perceived control and decreases in neuroticism) were mediated by changes in either mindfulness or purpose in life.

We made and tested a number of predictions about the associations between meditation training, psychological change, and telomerase activity: (1) The retreat group (compared to the matched control group) would exhibit greater post-retreat telomerase activity and show increases in mindfulness, purpose in life, and perceived control and larger decreases in neuroticism. (2) Group differences in post-retreat telomerase activity would be mediated by meditation-influenced increases in mindfulness, purpose in life, and perceived control, and decreases in neuroticism. (3) Meditation-induced increases in mindfulness and purpose in life would mediate retreat-related increases in perceived control and decreases in neuroticism.

2 Materials and methods

2.1 Study overview

Sixty men and women (aged 21–69) were matched on demographic variables and meditation experience (described below) and randomly assigned to either an on-site, three-month meditation retreat or a wait-list control group. All participants were assessed before and after the retreat with self-report measures (details below), and telomerase activity was assessed in both groups in

peripheral blood mononuclear cells (PBMC) obtained at the end of the retreat. Self-report measures were also administered in a subsequent follow-up. All samples and self-report measures were collected at the site of the retreat.

2.2 Participants

Participants were recruited nationally through advertisements displayed in meditation centers and Buddhist magazines and on Buddhist websites. The ad stated that the study's primary aim was to "investigate the relation between meditation and well-being." Interested individuals submitted applications and were screened (with a 50% acceptance rate) based on the following criteria: (1) age between 21 and 70; (2) an agreement to refrain from alcohol, tobacco, and recreational drug use during the retreat and from tobacco and recreational drug use in the 3 months prior; (3) availability at all testing points and the flexibility to be assigned to either the wait-list control group or the retreat group; (4) no serious medical or psychological problems; axis I psychiatric impairments were assessed by administering both the M.I.N.I. screen (Sheehan et al., 1998) and a brief clinical telephone interview, conducted by a licensed clinical psychologist; (5) previous participation in three or more short (5–10 day) meditation retreats, with at least one of them led by Alan Wallace, Ph.D., who led the retreat in this study. This last criterion ensured that participants knew what the retreat would entail and were unlikely to leave the study prematurely.

Stratified matched assignment was used to assign participants to either the retreat condition (n = 30) or the waitlist control condition (n = 30), with groups matched on sex (28 men and 32 women), age (M = 48, range 22–69), and years of self-reported meditation experience (M = 13) (Table 1 (tbl0015)).

Table 1

Group Matching: Demographics and Psychological Variables.

	Control	Retreat	t	p [j] (tblfn0120)
All Participants [a] (tblfn0075)				
General				
Age [c] (tblfn0085)	46 (22–65)	49 (23–69)	0.79	n.s
Sex	14 M, 16 F	14 M, 16 F	–	–
Education [d] (tblfn0090)	4.9 (3–6)	5.2 (1–6)	1.09	n.s
Income [e] (tblfn0095)	6.5 (1–11)	6.9 (1–11)	0.41	n.s
BMI [f] (tblfn0100)	23.6 (17.7–39.5)	23.7 (19.3–32.9)	0.34	n.s
Meditation Experience				

Retreats [g] (tblfn0105)	15 (2–100)	13 (2–50)	0.67	n.s
Daily [h] (tblfn0110)	54 (13–155)	56 (9–180)	0.18	n.s
Lifetime [i] (tblfn0115)	2,668 (200–15,000)	2,549 (250–9,500)	0.16	n.s
Sub-sample [b] (tblfn0080)				
General				
Age [c] (tblfn0085)	50 (22–65)	53 (24–67)	0.59	n.s
Sex	12 M, 13 F	10 M, 6 F	–	–
Education [d] (tblfn0090)	4.8 (3–6)	5.2 (1–6)	1.02	n.s
Income [e] (tblfn0095)	7.3 (1–11)	7.1 (1–11)	0.34	n.s
BMI [f] (tblfn0100)	23.1 (17.7–27.4)	22.9 (19.3–30.5)	0.06	n.s
Meditation Experience				
Retreats [g] (tblfn0105)	16 (2–100)	14 (2–50)	0.92	n.s
Daily [h] (tblfn0110)	55 (13–155)	52 (9–90)	0.19	n.s
Lifetime [i] (tblfn0115)	2,979 (200–15,000)	2,588 (400–8,720)	0.86	n.s

a All participants that participated in the study.

b Sub-sample of participants for which telomerase activity could be assessed.

c Age in years at the beginning of participation in the study.

d Scale of education achievement (1 = less than high school; 2 = high school degree; 3 = some college; 4 = college degree; 5 = some graduate; 6 = graduate degree).

e Income category (1 = under 10K, 2 = 10–20K, 3 = 20–30K, 4 = 30–40K, 5 = 40–50K, 6 = 50–60K, 7 = 60–70K, 8 = 70–80K, 9 = 80–90K, 10 = 90–100K, 11 = over 100).

f Body Mass Index (pre–retreat) = mass (lbs) × 703/height (in) ^{2} .

g Total number of meditation retreats lasting at least 5 consecutive days. Reports available for 28 wait-list control and 30 retreat participants.

h Average daily minutes of formal meditation practice. Reports available for 28 wait-list control and 25 retreat participants.

i Total lifetime hours of formal meditation practice. Reports available for 30 wait-list control and 29 retreat participants.

j $p > .2$ for all tests using one-tailed significance criteria for all.

Control participants were flown to the retreat center before, during, and after the retreat to be assessed on multiple measures along with retreat participants, and they were on-site for 5 days prior to PBMC sample collection to adjust to the setting and altitude. Otherwise, the controls spent the remainder of the retreat period at their own homes, living their usual daily lives. Retreat participants paid for their room and board during the retreat (~$5300), but were compensated for participation in our assessments at the rate of $20/h. Control participants were also compensated for their participation in assessments and additionally for travel to the retreat site. All procedures were approved by the institutional review board of the University of California, Davis and carried out with written consent and adequate understanding by all participants using a protocol.

2.3 Meditation training

The meditation retreat took place within an isolated retreat setting (the Shambhala Mountain Center in northern Colorado), where retreat participants lived and practiced meditation techniques for 3 months. They were instructed in these practices by Alan Wallace, Ph.D., a well-known Buddhist scholar and practitioner, who has described the practices used for this particular study in detail (Wallace, 2006). These practices can be broadly categorized as the cultivation of attentional skills and the generation of benevolent mental states.

Attentional skill practices involve focusing the mind. The mind is first calmed to reduce distraction (the practice of *mindfulness of breathing*), and then the meditator, within that calmness, is trained to be aware of moment-to-moment thoughts in a manner that is non-reactive and stable yet discerning and vivid (the practice of *observing mental events* or “settling the mind in its natural state”). Further, the nature of consciousness is sometimes explored by disengaging attention from one's thoughts and focusing on awareness itself as the object of concentration (using the practice of *observing the nature of consciousness* or “awareness of awareness”).

Generating benevolent mental states involves the following: Loving-kindness practices arouse a heartfelt wish that self and others may experience happiness and its true causes, which serves as an antidote for malice. *Compassion* practices arouse a heartfelt wish that self and others may be free from suffering and its true causes, which is intended to serve as an antidote for cruelty. Practices intended to increase *empathetic joy* arouse delight in one's own and other people's joys and virtues, which serves as an antidote for envy and cynicism. Finally, *equanimity* practices arouse an impartial and unconditional sense of affectionate concern for others, regardless of their relation to oneself, which serves as an antidote to self-centered attachment and aversion.

The meditation training group met with Dr. Wallace in the mornings and evenings for short guided meditations and discussions. For the remainder of the day, participants engaged in solitary meditation sessions, for an average of 6.3 h in total (*SD* = 1.34). Although the main focus of the retreat was the cultivation of attentional skills, with the generation of benevolent states playing an ancillary role, retreat participants were encouraged to explore all of the methods presented. Most of them then settled on two or three of the practices. Participants recorded the type and duration of meditation practice in daily logs. At a follow-up assessment, participants also estimated the amount of time they had devoted to any type of Shamatha practice since the end of the retreat (~5 months).

When participants were not engaging in the practices specified above, they were instructed to maintain peripheral attention on a chosen meditative object while being mindful of actions and sensations involved in daily activities such as walking and eating. Participants also met individually with Alan Wallace on a weekly basis for clarification or guidance.

2.4 Telomerase measurement

2.4.1 Blood sample collection, PBMC isolation, and extract preparation

For each participant, 10 ml of peripheral blood was collected and anticoagulated in BD Vacutainer® CPT tubes with density gradient polymer gel and sodium citrate additives. The PBMC fraction was isolated from each blood sample using density gradient centrifugation (3500 rpm, 20 min, 18–25 °C). Immediately following centrifugation, the PBMC layer was collected. Cells were then washed 3 times in phosphate-buffered saline (PBS) by centrifugation at 3750 rpm for 10 min at room temperature. Cells were re-suspended in PBS and live cells were counted with Trypan blue staining solution using 5 squares of a hemocytometer, each measuring 0.04 mm^2 . Using this cell count, 6.25 million PBMCs were pelleted and extracts corresponding to 31,250 cells/μL were made, based on the protocol provided in the TRAPeze telomerase detection kit (Chemicon, Temecula, CA). The extracts were stored at −80 °C until use.

2.4.2 Telomeric repeat amplification protocol

Quantification of telomerase activity was measured from the extract using the telomeric repeat amplification protocol (TRAP) as previously described (Kim and Wu, 1997) with a commercial kit (TRAPeze®, Chemicon, Temecula, CA). Between 15,625 and 31,250 cells were used for TRAP reactions to ensure that the assay was in linear range for each sample (Lin et al., 2009a). The reaction was carried out according to the TRAPeze kit manual. The PCR program used was: 94 °C for 2 min; 94 °C for 30 s, 59 °C for 30 s for 30 cycles. The products were fractionated on a 10% polyacrylamide-8 M urea sequencing gel. The gel was exposed to a phosphorimager plate overnight and scanned on a STORM 860 molecular imager (GE Healthcare, Piscataway, NJ). The 293T cell line was used as a positive telomerase activity control and reference standard.

Telomerase activity was quantified using the software ImageQuant 5.2 (GE Healthcare, Piscataway, NJ). Signals from the product ladders on the gels were added and normalized against the signal from an internal control band for the same lane to get the product/internal control value. For each telomerase activity assay reaction, the product/internal value was divided by the product/internal control value from twenty 293T cells and then multiplied by 20 to obtain the final telomerase activity units, defined as 1 unit = the amount of product from one 293T cell/15,625 PBMCs. The inter-assay variability (CV) was 6.7%. After obtaining values of telomerase activity for 15,625 cells, a linear correction was applied whereby values were multiplied by 0.64 so that results were comparable with previous findings, which typically report activity per 10,000 cells.

2.4.3 Criteria for assessing telomerase activity

Within the groups of retreat and control participants, eligibility for telomerase activity assessment included (1) consent to have a blood sample drawn; (2) an adequate number of peripheral blood mononuclear cells (PBMCs) from the pre-processed blood sample; (3) no self-reported illness on the day of (or surrounding) blood sample collection; (4) no self-reported, preexisting health conditions; (5) a body mass index (BMI) that did not indicate morbid obesity (BMI above 40); (6) no use of medications that could potentially affect telomerase activity derived from PBMCs.

Fourteen participants either declined to have their blood drawn (2 participants) or their blood samples contained inadequate numbers of PBMCs (12 participants), reducing the assayed sample size to 26 controls and 20 retreat participants. Of the assayed samples, one participant in the control group had a BMI > 40, indicating morbid obesity. Within the retreat group, one participant reported taking fertility treatments during the study, one reported a liver disorder, and one reported cancer remission and was taken to a medical facility during the retreat for an unrelated stomach ulcer. This reduced sample sizes *for models that included telomerase* in the retreat group (to $n = 17$) and control group (to $n = 25$). Within this reduced sample, demographic variables and prior meditation experience did not differ significantly between retreat and control groups (Table 1 (tbl0015)). Additionally, within the treatment group, the group of participants who were not included in the analyses did not significantly differ from the remaining 17 subjects on any of the pre-treatment baseline assessments. That is, independent sample t -tests comparing the included vs. excluded participants were not significant for pre-retreat Purpose in Life ($t = 0.14$, $p = 0.89$, two-tailed), Mindfulness ($t = 1.30$ $p = 0.27$, two-tailed), Neuroticism ($t = -0.71$, $p = 0.49$, two-tailed), or Perceived Control ($t = 1.61$, $p = 0.12$, two-tailed) (Although not significant, the mean value for pre-retreat Perceived Control was slightly lower in the group that was not included in the analyses.).

2.5 Psychological measures

Psychological assessments were completed at pre and post-retreat time-points. At the post-retreat time-point, assessments were completed within 2 days prior to blood sample collection. Follow-up at home psychological assessments were completed for the retreat group at ~5 months following the

retreat. All measures contained items that were rated on a 7-point scale, ranging from 1 (*Disagree Strongly*) to 7 (*Agree Strongly*). Two participants did not complete any of the pre-retreat psychological assessments, and an additional two did not complete the Mindfulness measure. One participant did not complete any of the follow-up assessments and an additional participant completed all follow-up assessments except the Mindfulness measure. These participants were not included in analyses involving those measures. The internal reliability indices (Cronbach's alpha) of the measures and their intercorrelations are reported in Table 2 (tbl0010) .

Table 2

Partial correlations between psychological measures in the combined control and retreat groups (controlling for age).

Pre-retreat	*α*	Mindfulness	Purpose	Control
Mindfulness	0.95 [a] (tblfn0055)	–		
Purpose	0.74	0.29 * (tblfn0060)	–	
Control	0.87	0.41 ** (tblfn0065)	0.62	–
Neuroticism	0.86	–0.48 *** (tblfn0070)	–0.44 *** (tblfn0070)	–0.68 *** (tblfn0070)

Δ(Post − pre)	ΔMindfulness	ΔPurpose	ΔControl
Mindfulness	–		
Purpose	0.25 * (tblfn0060)	–	
Control	0.37 ** (tblfn0065)	0.55 *** (tblfn0070)	–
Neuroticism	–0.42 ** (tblfn0065)	–0.35 ** (tblfn0065)	–0.49 *** (tblfn0070)

α = Cronbach's alpha at pre-retreat.

a Entire scale averaged (individual facets ranged from 0.80 to 0.93).

* $p < 0.05$ (all one-tailed criteria).

** $p < 0.01$ (all one-tailed criteria).

*** $p < 0.001$ (all one-tailed criteria).

2.5.1 Mindfulness

Participants completed the 37-item Five Facet Mindfulness Questionnaire (FFMQ), which assesses five facets of Mindfulness (observing or noticing experience; acting with attentional awareness or avoiding automatic pilot; non-reactivity to internal experience; describing or labeling feelings; non-judging of experience) (Baer et al., 2006). The facets of the FFMQ were intercorrelated, and a factor analysis indicated that a single factor accounted for 55% of the variance in the facet scores, which loaded on the factor with values ranging from 0.65 to 0.84. We therefore used a single score for mindfulness, the mean of all the item scores.

2.5.2 Purpose in life and perceived control

Ryff's (1989) Well-Being Scale assesses how a person rates various aspects of his or her functioning on six dimensions, one of which is Purpose in Life and another of which is Environmental Mastery (or, in our terms, Perceived Control). We used the 9-item Purpose in Life subscale to assess changes in a person's sense that life is meaningful, organized around clear aims, and clearly directed. We used the 9-item Environmental Mastery subscale to measure Perceived Control over situations and circumstances.

2.5.3 Neuroticism

The Big Five Inventory (John et al., 1991; John and Srivastava, 1999) is a simple measure of the five major broadband personality factors derived from intensive study of personality structure over the past few decades. We used the 8-item Neuroticism scale to assess dispositional negative emotionality. Higher scores indicate being relatively tense, moody, and anxious.