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Monica Isgut April 14th, 2015

Pilot Study on the Feasibility of a Meditation Intervention to Change Telomere Length in College Students

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Abstract

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Telomeres are heterochromatic nucleoprotein structures at the ends of chromosomes that protect them from end-fusion and degradation. They shorten with each cell division due to the end-replication problem, and ultimately reach a critical length at which point they are recognized as double-stranded breaks and the cell enters a state of cell-cycle arrest. Telomeres can be elongated primarily by an enzyme called telomerase, which in adult tissues is expressed mostly in stem cells. Telomere shortening is associated with cellular senescence, age-related tissue dysfunction, and the onset of morbidity and mortality in humans. In the long term, telomere length in an individual has generally been shown to shorten consistently with age, but short-term longitudinal studies show that telomere length tends to fluctuate when studied over the course of a few months or years. Psychological stress seems to be associated with shorter telomeres. This may be due to the effects of psychological stress on oxidative stress and inflammatory pathways, which in turn affect telomere length dynamics. It was of interest in this study whether meditation, which has been shown empirically to reduce psychological stress and increase telomerase activity, could have an impact on telomere length. This research examines the effects of a one-month meditation intervention on measures of psychological health and telomere length in college students, and aims to provide the basis for a more large-scale study in the future.

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Introduction

Telomere Structure, Function, and Regulation

Telomeres are nucleoprotein structures at the ends of chromosomes that function to protect chromosomes from end-fusion and degradation (Houben et al., 2008). They consist of a series of around 4 to 15 kbp of 5'-TTAGGG-3' repeats, and have a G-rich overhang of about 100-150 nucleotides beyond the complementary strand (Houben et al., 2008). The G-rich strand is protected from degradation by intercalating into double-stranded telomeric DNA and forming what is called a T-loop (Houben et al., 2008; Blasco, 2007). Telomeric nucleic acids interact with a host of proteins, multiple of which are part of the shelterin-telosome complex and bind to the G-rich overhang and/or the double-stranded region (Blasco, 2007). These proteins play major roles in telomere length regulation and chromosome end protection, and are also functionally associated with proteins involved in DNA damage repair pathways (Houben et al., 2008). Additionally, telomeres contain high levels of H3K9 (histone 3, lysine 9) and H4K20 (histone 4, lysine 20) tri-methylation, and are thus constitutively heterochromatic (Blasco, 2007).

Telomeres tend to shorten with each cell division due to the end-replication problem inherent to DNA replication (Houben, et al., 2008). The end-replication problem occurs because during replication of the lagging strand after the last RNA primer has been removed, DNA polymerase is unable to bind to the strand to fill the gap at the end of the chromosome (Houben, et. al, 2008), leaving the G-rich overhang and a shortened new strand. When telomeres shorten substantially, they reach what is called the Hayflick Limit (Shalev, et al., 2013). Chromosome ends are recognized as double-stranded breaks and this activates proteins involved in DNA damage repair pathways, such as cyclin-dependent kinase inhibitor 1A (p21), which leads to cell-cycle arrest (Shawi et al., 2008). If the cell cycle is not restored, the cell enters a state of senescence, which is characterized by arrested reproduction, loss of ability of recognize antigens,

a higher than typical volume, changes in gene expression and metabolism, and the increased secretion of pro-inflammatory cytokines (Shawi et al. 2008; Puterman et al, 2012; Shalev, 2012).

Telomeres can be elongated, primarily by an enzyme called telomerase (Houben, et al., 2008). Telomerase consists of two molecules of a reverse transcriptase domain (Tert), two molecules of an RNA template (Terc), and one molecule of a protein that holds it together (dyskeratin) (Blasco, 2007). Telomerase uses its template RNA to elongate the G-rich overhang so that it can be used as a template for further replication of the other strand (Houben et al., 2008; Blasco, 2007). In humans, this enzyme is expressed at high levels during embryonic development, but expression is down-regulated and minimal in most adult somatic cells after birth (Blasco, 2007). Stem cells are some of the only adult cells that express telomerase (Blasco, 2007), and most somatic cell telomeres tend to continually shorten over time, ultimately leading to a state of cellular senescence.

Telomeres, Aging, and Cancer

A multitude of studies have shown telomere length to be strongly associated with human health and disease. Telomere-induced cellular senescence is considered to be an important contributing factor to age-related tissue dysfunction, reduced regenerative capacity, and the onset of morbidity and mortality in humans (Boccardi et al., 2014; Epel, et al., 2004). Senescent cells tend to accumulate in the skin, retina, liver, and other tissues over time (Shawi, 2008), and this accumulation is thought to be due to the decreased ability of stem cells to regenerate those tissues as an organism ages (Blasco, 2007). One theory of aging suggests that the mobilization of stem cells to tissues in need of repair is decreased in stem cell niches with short telomeres and senescent cells (Blasco, 2007), and that this promotes tissue dysfunction. Short telomeres have

been shown to be associated with risk of dementia, cognitive impairment and heart disease, and with incidence of acute myocardial infarction (Blasco, 2007; Epel, 2004; Thomas et al., 2008).

In contrast, long telomeres and telomerase overexpression have been associated with cell immortalization in cancerous tumors (Shawi et al., 2008). Short telomeres have been shown to have an important tumor-suppressor function by triggering tumor protein p53-dependent pathways which ultimately lead to cellular senescence and apoptosis in response to DNA damage (Shawi et al., 2008). Thus, telomeres seem to have a pleiotropic effect in humans by acting as tumor suppressors to prevent cancer early in life, but contributing to aging later in life (Shawi et al., 2008).

Telomere Length Homeostasis

Cellular proliferation seems to be the main mechanism of telomere shortening (Houben et al., 2008), however oxidative stress and inflammation also seem to play an important role. Telomeres are particularly sensitive to damage by reactive oxygen species (ROS) due to their high guanine content, which allows for the formation of 8-oxo-dG base modifications (Houben et al., 2008). DNA damage repair is less efficient in telomeres than elsewhere in the genome, and this allows for the accumulation of base modifications over time (Houben et al., 2008). Unrepaired base modifications in DNA may interfere with the replication fork, possibly stopping replication early (Houben et al., 2008), and this may be a mechanism through which oxidative stress may cause telomere shortening. The presence of base lesions has also been shown to inhibit telomerase activity and to decrease binding of shelterin proteins involved in telomere length regulation and damage protection (O'Callaghan et al., 2011; Epel, 2009). Inflammation may also contribute to telomere shortening (Houben et al., 2008). For example, tumor necrosis

factor alpha (TNF-α) has been shown to down-regulate telomerase activity (Lin et al., 2012; Houben et al., 2008). Additionally, shorter telomeres have been associated with higher levels of the pro-inflammatory proteins interleukin 6 (IL-6) and C-reactive protein (CRP) in hemodialysis patients (Epel, 2009). Furthermore, inflammation is linked to the increased proliferation of immune cells, and this may lead to telomere shortening (Shalev, 2012).

While telomere elongation in humans occurs mainly due to the action of telomerase (Blasco, 2007), another mechanism, called alternative lengthening of telomeres (ALT), has also been shown to cause lengthening. This mechanism requires cells to have both long and short telomeres, and consists of homologous recombination events between telomeres of sister chromatids (Blasco, 2007). However, this is not a major mechanism in humans given that telomeric proteins and methylation patterns seem to exert strong regulatory control against this type of damage (Blasco, 2007).

Overall, telomeres are in constant homeostasis, and while they may shorten over extended periods of time during an individual's life, they have also been shown to lengthen when studied longitudinally over short periods of time (Epel, 2012). Telomere length tends to vary between tissue types in an individual, due to differences in cell turnover rates, stem cell capacity to regenerate or differentiate, exposure to oxidative damage, or the differential regulation of telomere length (Shalev, 2012). Telomere shortening rate varies between individuals. For example, the rate seems to be higher in males than in females (Blasco, 2007) and it seems to vary by age (Epel et al., 2004). One long-term longitudinal study of telomere length in the white blood cells of a cohort of women has shown that telomeres decreased by an average of about 27 base pairs each year (Shawi et al., 2008). Another study on telomere shortening in peripheral blood mononuclear cells (PBMC's) suggests that telomeres seem to shorten at a minimal rate in

young adulthood but at a faster rate (of approximately 60 base pairs each year) between the ages of 50 and 70 (Epel et al., 2004). Telomere lengths in post-mitotic tissues such as nerve, skeletal muscle, and bone tissue, change little over time (Price et al., 2013). In a given individual, telomere length seems to depend on the initial setting of telomere length before birth, and subsequently the individual's exposures after birth (Shalev et al., 2013). The heritability of telomere length has been estimated to be around 34-82%, and before birth, telomere length may be influenced intrauterine conditions (Shalev et al., 2013).

Psychological Stress and Telomere Length

Psychological stress has been shown to be an important pathway through which an individual's life experience may influence their telomere length (Epel et al., 2009). The link between stress and telomere length has been examined in multiple studies. Early life stress seems to have a significant impact on telomere length (Shalev et al., 2012, Lin et al., 2012). A longitudinal study on childhood exposure to violence between the ages of 5 and 10 showed that children who had been subject to violence during that time had significantly shorter telomeres at the end of the study than those who did not experience violence (Shalev et al., 2013). Additionally, childhood trauma seems to predict short telomeres in adult cells (Shalev et al., 2013). However, chronic adult stress also seems to be deleterious. One study found that mothers who were caregivers for chronically ill children had significantly shorter telomeres the longer they had been exposed to the stressor (Lin et al., 2012). Another study on female caregivers showed that women with higher anticipatory threat ratings before a laboratory stressor had significantly shorter telomeres than those who had higher challenge ratings (O'Donovan), and this suggests that an individual's perception of a stress rather than the stressor itself may be the important factor associated with telomere shortening.

An individual's emotional reaction to a stressor depends on his or her cognitive appraisal of the situation (Kemeny, 2003; Epel, 2009), which in turn is based on the individual's personal vulnerability to the stressor (Dickerson et al., 2004). Threat appraisals to a situation, in which a stressor is perceived to be out of the individual's control, induce negative emotional responses (Epel, 2009). In contrast, challenge appraisals, which involve a sense of control over the situation, are associated with more positive emotions (Epel, 2009). A stressful event typically affects the hypothalamic-pituitary-adrenal (HPA) axis (Kemeny, 2003), which culminates in the release of cortisol into the bloodstream and the subsequent onset of the "fight or flight" response which is associated with heightened activation of the sympathetic nervous system (Dickerson et al., 2004). When stress becomes chronic, this may lead to dysregulation of the HPA axis which may lead to a blunted diurnal rhythm of cortisol or to elevated base levels (Epel, 2009). It may also contribute to the suppression of certain anabolic hormones and to a depressed vagal tone (Epel, 2009). Chronic stress may lead to increased levels of reactive oxygen species (Epel et al., 2004) and inflammation (Miller et al., 2008), which in turn may induce telomere shortening.

The mechanistic link between chronic psychological stress, oxidative stress, and inflammation is worth noting. While cortisol typically plays a role in the down-regulation of pro-inflammatory cytokines, these inhibitory mechanisms are blunted when base cortisol levels are consistently elevated, leading to increased levels of systemic inflammation (Miller et al., 2008). In addition to inflammation, chronic stress may also lead to localized and systemic damage by reactive oxygen species (ROS). The stress response increases levels of calcium and of the excitatory neurotransmitters glutamate and aspartate, which when chronically elevated may lead to excitotoxicity in neurons (Liu, 1996). Excitotoxicity involves excessive levels of calcium in affected neurons, and this leads to the opening of mitochondrial transition pores, which may

cause mitochondria to swell and release reactive oxygen species in the neurons (Stavrovskaya et al., 2005). Systemic oxidative stress refers to the imbalance between oxidants and antioxidants in the body (Cerda et al., 1997), and is intricately linked to the inflammatory response. The inflammatory response mobilizes neutrophils and macrophages, which release reactive oxygen species through the process of respiratory burst as part of their defense mechanisms (Kang, 2002). In turn, reactive oxygen species may lead to cell damage and the up-regulation of certain molecules which promote the inflammatory response (Salvemini et al., 2006). Thus, when stress ensues over a long period of time, not only does it lead to consistently elevated inflammation and oxidative stress, but it also allows the two to reinforce each other via their interrelated pathways, leading to an even greater overall effect.

Mindfulness Meditation and Telomere Length

Given the detrimental effects of psychological stress on telomere length, it is of interest whether lifestyle interventions that help with stress management could have an effect on telomere length and rate of shortening. Mindfulness meditation training could be one such potential intervention (Epel et al., 2009). Mindfulness is a state of mind that consists of focusing attention on the present moment without judgment and without engaging with unrelated thoughts, beliefs, or emotions (Epel et al., 2012). The goal during mindfulness meditation training is to notice fully every thought, sensation, or emotion that arises, and to "let go" of those distractions and return attention to the breath or to an object of focus (Epel et al., 2009). Ultimately, perhaps with sufficient practice, it is possible for mindfulness skills cultivated during meditation training to become a part of an individual's daily life outside of practice time (Epel et al., 2009).

There are multiple cognitive processes through which cultivating mindfulness may reduce stress and promote a state of psychological well-being. Mindfulness improves attention to present-moment experiences, and this may help reduce ruminative thought which is considered to be associated with increased reactivity to stressors (Epel et al., 2009). Mindfulness is thought to reduce the extent to which stressors are appraised as threatening, and it may help with coping simply by increasing an individual's sense of perceived control over a situation (Epel et al., 2009). Finally, mindfulness is thought to assist in emotional regulation and to increase the frequency and intensity of positive emotions such as empathy, kindness, and compassion (Epel et al. 2009) as well as in feelings of self-acceptance, positive social relations, autonomy, environmental mastery, purpose in life, and personal growth (Oman et al., 2008).

The associations between mindfulness meditation training, decreased psychological stress, and improved psychological well-being provide a foundation from which to hypothesize that meditation training could have a significant effect on telomere biology in humans. Since meditative practice is beneficial in reducing psychological stress, and since psychological stress is linked to high levels of inflammation and oxidative stress which are associated with shorter telomeres, perhaps meditation could attenuate stress-induced telomere shortening. This would have implications on the rate of cellular senescence and tissue aging. Moreover, it is important to note that meditation also induces a state of psychological well-being, which is associated with positive emotions and the activation of anabolic hormones that have been shown to promote telomerase activity (Epel et al., 2009). It is not surprising that a 3-month longitudinal study by Jacobs et al. on meditation retreat participants found that telomerase activity in retreat participants was significantly higher than controls post-intervention (Jacobs et al., 2011).

Another study on long-term female practitioners of loving-kindness meditation had significantly longer telomeres than control subjects (Hodge et al., 2013).

Aims and Hypothesis

The present study aims to test the aforementioned hypothesis on a cohort of college students over the course of a one-month mindfulness meditation intervention. We hypothesize that meditation will decrease levels of perceived stress and increase levels of psychological well-being. Additionally, we hypothesize that over the course of the intervention, meditation participants will have either a) more telomere elongation than controls or b) less telomere shortening than controls. We also hypothesize that there may be a negative correlation between changes in psychological stress levels and changes in telomere length, and a positive correlation between changes in psychological well-being and changes in telomere length.

Materials and Methods Section

Participants

This research has been approved for the study of human subjects by the Emory Institutional Review Board (IRB) on January 27th, 2015. All study participants were Emory University undergraduate students. The intervention subjects were recruited at the information session for a four-week introductory mindfulness meditation class taught by two instructors from the Atlanta Shambhala Meditation Center. The controls were recruited via an email sent out to students at a residential center on the university's campus, as well as by word of mouth. Students were excluded from the study if they were already experienced in meditation, if they were taking anti-inflammatory medications, if they abused drugs or alcohol, and/or if they had any underlying medical conditions, such as asthma or clinical depression. While there was not a questionnaire at onset to determine eligibility to participate, subjects were verbally asked to participate only if they were eligible based on the exclusion criteria.

Originally, 10 control participants and 18 meditation participants signed consent forms to join the study, but subsequently six of the meditation participants dropped out of the study and were excluded. The final numbers of control and meditation participants were 10 and 12, respectively. The two groups are relatively similar in terms of gender distribution. While the mean age of both groups differed by less than one year, this difference was determined to be statistically significant ($p=0.02$; see Table 1). At study onset, there was no significant difference in Perceived Stress Scale score ($p=0.09$), Flourishing Scale score ($p=0.73$), and relative telomere length ($p=0.90$) between the intervention and control groups (see Table 1).

Table 1: Subject Demographics at Study Onset

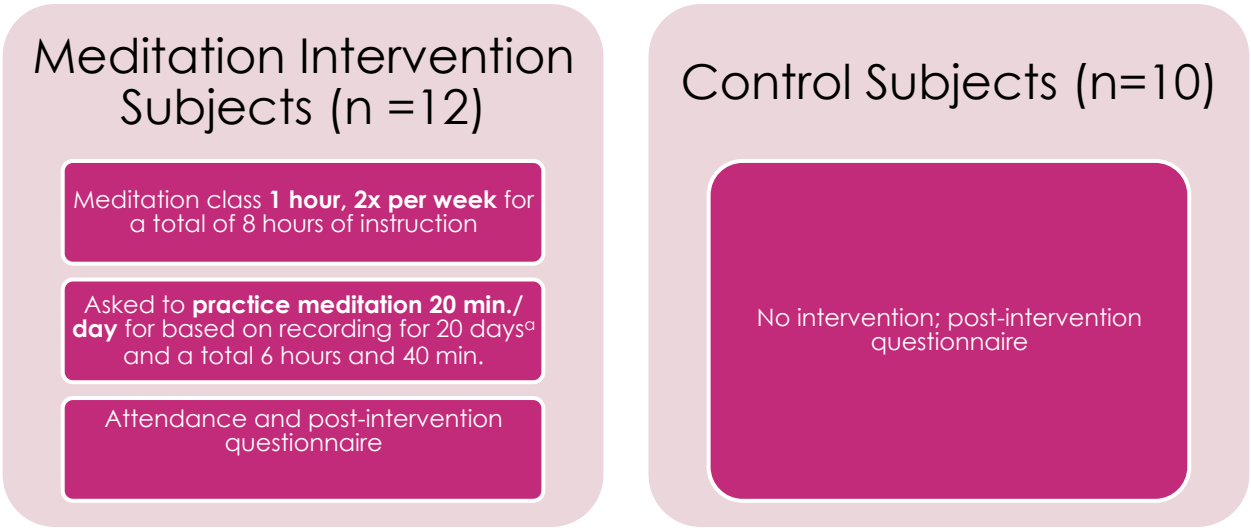
	Meditation (n=12)	Control (n=10)	p-value
Males	5	5	N/A
Females	7	5	N/A
Mean Age	20.33 (± 0.26)	21.20 (± 0.20)	0.02
Mean Initial PSS Score[a]	20.00 (± 1.99)	15.50 (± 1.28)	0.09
Mean Initial FS Score	44.08 (± 2.67)	45.40 (± 2.59)	0.73
Mean Initial RTL[b]	2.30 (± 0.23)	2.36 (± 0.30)	0.90

[a]*Excludes 1 missing data point for meditation subjects*
[b]*RTL = relative telomere length (T/S ratio); excludes 2 missing data points, one for controls and one for meditation subjects*

Intervention and Study Design

The intervention studied consisted of a four-week (28-day) introductory mindfulness meditation class meeting for one hour twice weekly at a multi-purpose classroom at the Woodruff Physical Education Center at Emory University, and additional daily outside practice. Full compliance to the intervention involved attending all 8 sessions, which consisted mostly of instruction with around 20 minutes of practice, and outside practice of 20 minutes daily five days per week based on a recording (see Figure 1). The recording consisted of a guided 20-minute mindfulness meditation session led by one of the instructors teaching the class.

Buccal swab samples and the psychological questionnaires were collected from the intervention participants three times: at the beginning of the first meditation session, after exactly two weeks, and again at the end of the intervention after two more weeks. The samples and questionnaires for control subjects were taken on the same days as those for the intervention participants. If intervention subjects did not attend sessions at the days of sample collection, or if control subjects were unable to meet, they were contacted and most were able to meet a few days later. The times of collection for these samples differed between participants depending on their availabilities.



[a] *20 days of home practice + 8 days of instruction = 28 days total*

Figure 1: **Study Design Visual**

DNA Collection, Extraction and Purification

The buccal samples were collected with commercially available Catch-All™ Sample Collection Swabs. Study participants were instructed to wear gloves and rub and rotate the swab against the inside of both cheeks for around 1-2 minutes in total, after which each swab was placed on the open wrapper for about five minutes to dry before it was placed inside the wrapper and sealed with tape. Samples were kept in a portable cooler until they were taken to the laboratory and stored in a -20°C freezer.

The DNA extraction followed a standard protocol, and was done in batches of 4-8 samples. The buccal swabs were removed from their wrappers and each was rubbed against a 2 mL Eppendorf™ tube containing 500 µL of digestion buffer (1M Tris-HCl pH 8.0, 0.5M EDTA, 20% SDS, 1:4:1 ratio) in order to remove cells from the swab. The buffer solution was used to help deactivate metal-dependent enzymes and to assist in cell lysis. After that, 12.5 µL of proteinase K solution was added to each tube, and they were incubated in a water bath at 56°C

for 30 minutes. Then 500 µL of Phenol:choloroform:isoamyl alcohol 25:24:1 saturated with 10mL Tris, pH 8.0, 1 mM EDTA (Sigma Aldrich™ P2069) was added to the tube and the solution was vigorously shaken until it appeared milky white. It was then incubated at room temperature for five minutes, and then centrifuged at 12,000x for 10 minutes at 4°C. After centrifugation, the aqueous solution of each tube was transferred to a new tube and the organic phase was discarded. Then 500 µL of isopropyl alcohol was added to the aqueous phase solution, and it was mixed by shaking vigorously. The solution was then incubated at room temperature for 5 minutes, and centrifuged at 12,000x for 15 minutes 4°C. The supernatant was removed by aspirating slowly with the pipette, and then 1 mL of 70% ethanol was added to the tube before another round of centrifugation of 5 minutes at 4°C. The alcohol was then removed and the tube was air dried for 45 minutes. Then 100 µL of Tris-EDTA (TE) buffer was added to the tube and it was incubated in a water bath at 50°C for 10 minutes. The final concentration of DNA for each sample was measured using the Thermo Scientific™ Nanodrop 2000® spectrophotometer (see Appendix A). Samples were stored overnight in a -20°C freezer.

After DNA extraction, the samples were purified using a standard laboratory procedure for ethanol precipitation of nucleic acids. First, 2 µg of DNA in solution of each sample was pipetted into a 96-well plate, with a different volume in each well depending on the concentration of DNA in each sample extracted previously. The 96-well plate was then placed in a Thermo Scientific™ Savant DNA 120 SpeedVac Concentrator® machine for as long as necessary to evaporate all of the volume in each well. Once the fluid was removed from the plate, 1.9 µL of 3M sodium acetate and 60 µL of 85% ethanol were added to each well, and the plate was vortexed briefly and centrifuged for 45 minutes at 4000 rpm and 4°C using an Eppendorf™ Centrifuge 5810 R® 15 amp version. Then the supernatant was removed and

inverted with paper towels, and 150 µL of 70% ethanol solution were added to each well. The plate was centrifuged again, for 15 minutes at 4000 rpm and 4°C. The plate was then placed in the SpeedVac Concentrator® machine again for 30 minutes, after which 100 µL of water were added to each well. The plate was sealed and then stored in a 4°C refrigerator.

Measures

- *Relative Telomere Length (RTL)*

Relative telomere length was the only biological measure in the study. Relative telomere length was measured using the quantitative PCR (qPCR) method in reference to the 36B4 standard gene length, and the result was a telomere to single-copy gene (T/S) ratio. The procedure consisted of two PCR reactions using a Life Technologies™ ViiA 7® machine. One reaction used telomere primers and the other used 36B4 single gene primers. The telomere qPCR primer solutions were 1µM telg (GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT) and 1 µM telc (TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA). A master mix was made using distilled water, the telg and telc primer solutions, and 1x PerfeCTa™ SYBR Green SuperMix ROX™ (CAT No. 95055-100), and this was pipetted into a 364-well plate with 100 ng of template DNA from each participant. The PCR reaction ran at 95°C for 5 minutes, then cycled 30 times at 95°C for 15 seconds and 54°C for 2 minutes, after which the temperature was held at 4°C. The 36B4 single-gene PCR reaction used 1 µM 36B4u (CAGCAAGTGGGAAGGTGTAATCC) and 1 µM 36B4d (CCCATTCTATCATCAACGGGTACAA) primers. A master mix was prepared with distilled water, both single-gene primers, and the mentioned 1x SYBR Green SuperMix ROX™ solution, and it was pipetted into a 364-well plate with 100 ng of template DNA. The PCR reaction was

held at 95°C for 5 minutes, and then cycled 30 times at 95°C for 15 seconds and 60°C for 1 minute, followed by a resting temperature of 4°C. Both qPCR reactions contained one non-template control (NTC) sample and six standards containing 120 ng, 60 ng, 30 ng, 15 ng, 7.5 ng, and 3.75 ng, respectively, of reference DNA. All samples for each of the reactions were performed in duplicate in the same plate. The resulting T/S ratio was determined by dividing the average quantity measure of telomere length by the average quantity measure of the single-gene copy length for each sample. Samples were excluded from final data analysis if a) the resulting T/S ratio was an outlier greater than 2.5 standard deviations away from the mean, or b) there were no assay results (ex. the quantities for either or both of the reactions was below threshold and did not get measured).

- *Psychological Questionnaires*

The participants' appraisal of the extent to which situations in their lives over the previous month were considered overwhelming, unpredictable, or uncontrollable was measured using the 10-item Perceived Stress Scale (PSS) (Cohen et. al, 1983). Participants' appraisal of their self-esteem, purpose in life, level of optimism, and perceived success in relationships was measured using the 8-item Flourishing Scale (FS) (Diener et. al, 2010). Both questionnaires have been validated previously (Cohen et. al 1983, Diener et. al, 2010, Silva et. al, 2013), and measures were taken from participants at all three time points in the study. The PSS and FS questionnaires are in Appendix B and Appendix C, respectively.

- *Compliance and Confounding Factors (post-completion questionnaire)*

Attendance was taken at each meditation session. Compliance to outside practice was originally attempted to be recorded by weekly logs, but not all students returned logs each session, and even if logs were asked to be completed retrospectively for the week during the

sessions, not all students attended all sessions, which led to incomplete data. Thus, participant compliance was measured retrospectively using a post-completion questionnaire, which participants were asked to estimate how many days per week on average they practiced 20 minutes of meditation in addition to the twice-weekly sessions.

In addition to compliance, the post-completion questionnaire asked participants to rate the helpfulness of the intervention in reducing their level of stress, from 1 (being the least helpful) to 10 (being the most helpful).

All participants were given a post-completion questionnaire, but controls were exempted from completing the intervention-related questions. The other questions asked participants whether they had experienced a stressful life situation over the previous month, and if so, they were asked to rate it from 1 (being the least stressful) to 10 (being the most stressful) and to state whether they perceived the stressor as more challenging or more threatening after being explained the differences (See Appendix D). While those questions were meant to assess whether there may be confounding factors in the participants' lives that could influence the results, they have not been validated.

Compliance Measures

A composite score for overall compliance to the intervention (both attendance and outside practice) was used in the data analysis. Attendance was counted as one "day" of 20-minute meditation practice, and the retrospective data from the post-completion questionnaire was used to measure approximate amount of outside practice. The four options for weekly outside practice on the questionnaire were: a) practiced every day or almost every day, b) practiced an average of 3-5 days per week, c) practiced an average of 1-2 days per week, d)

practiced on average less than once per week. The average of the range of days per week the subjects claimed to have practiced was taken and multiplied by the number of weeks (four) of the meditation class, and this was added to the attendance count. This was expressed as a percentage of the total number of days of practice assuming perfect compliance to the intervention (28 days). If the subjects picked (d), only attendance was included in the final compliance measure (See Appendix D). The compliance score is given as a decimal from 0-1.

Statistical Analysis

The main questions in the study were whether subjects in the meditation class would experience lower perceived stress and improved psychological well-being after the intervention than before, whether intervention subjects had less telomere shortening than control subjects over the course of the study, and whether these changes were correlated with compliance to meditation practice. Changes in PSS scores, FS scores, and RTL values after the intervention were analyzed for statistical significance in meditation and control subjects using a two-tailed t-test, and likewise for differences in PSS scores, FS scores, and RTL values at onset and at the end of the study between the two groups. Correlations between compliance scores and the extent of post-intervention changes in PSS scores, FS scores, and RTL were analyzed with linear regression models and statistical significance was tested using the Pearson's correlation test with r (correlation coefficient) values and a two-tailed p-value. All p-values of $p \leq 0.05$ were considered to be statistically significant.

In addition, we studied the general correlations between changes in telomere length, perceived stress, and flourishing in all subject. We also studied the cross-sectional associations between the three parameters in samples taken from all subjects for multiple time points at once, to determine the psychological questionnaires may be good indicators of telomere length at any

one time point. These correlations were tested for statistical significance using a Pearson's correlation test.

Results

Attendance, Overall Compliance, and Confounders

Since this research studies the effects of a lifestyle intervention, it was important to have a measure for attendance and overall compliance. The mean meditation class attendance was 46.9% (± 17.8%), or approximately 3.75 out of a total of 8 classes. Overall class attendance decreased over the course of the study period (See Figure 2). The mean compliance score, which combined total attendance with outside practice, was 0.53 (± 0.07) out of 1 (See Table 2). The mean helpfulness rating was 5.09 (± 0.75). Of note, female participants as a group tended to have significantly higher compliance than males ($p = 0.02$; See Table 2). Attendance was higher in the first half of the intervention than in the second half (see Appendix E). The post-assessment questionnaire (see Appendix D) included questions about life stresses which may be confounding factors in the study (see Appendix E for data). There was an approximately equal number of individuals in the meditation and control groups who claimed to have had a stressful life experience during the time of the study, and only one individual in each group perceived the event as threatening. The average rating of the intensity of the stressor for those who claimed to have experienced a stressful life event was significantly higher in the meditation group ($p=0.01$), which may have been a confounding factor in the study.

Table 2: Compliance Scores and Helpfulness Ratings

	Mean Compliance Score	**Mean Rating**
All Subjects (n=11)	0.53 (± 0.07)	5.09 (± 0.75)
Males (n=4)	0.34 (± 0.12)	3.75 (± 0.65)
Females (n=7)	0.63 (± 0.05)	5.86 (± 1.02)

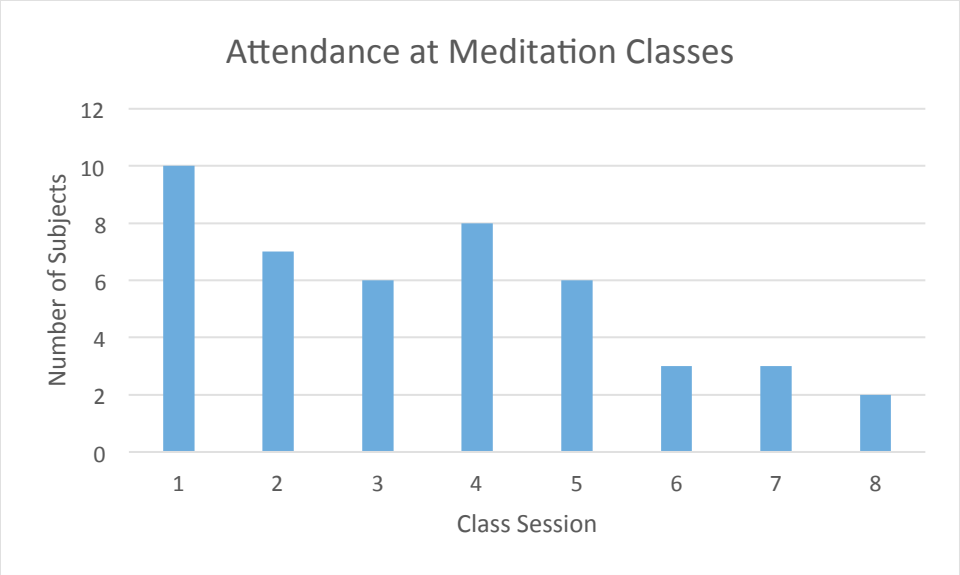


Figure 2: *Attendance at meditation classes, by number of students per class*

Perceived Stress and Flourishing

One of the main questions of the study was whether intervention subjects would experience lower levels of perceived stress and improved levels of psychological well-being after the four-week meditation class, and whether these changes in psychological parameters had any correlation with compliance score and/or with participants' ratings of the helpfulness of the class.

As hypothesized, the mean Perceived Stress Scale (PSS) score of the meditation subjects after the intervention decreased significantly over the course of the intervention ($p=0.03$) by 5.09 units (See Table 3, Figure 3C). There was no significant change in perceived stress observed in the control subjects. However, the pre- ($p=0.10$) and post- ($p=0.37$) mean PSS scores were not significantly different between meditation and control subjects (See Figure 3A). There seemed to be positive correlations between compliance scores and PSS scores ($r=0.33$), and between helpfulness ratings and PSS scores ($r=0.39$), but these correlations were not statistically significant ($p=0.36$ and $p=0.27$, respectively; See Figure 4A and 4C).

It was also hypothesized that the mean Flourishing Scale (FS) score of the meditation subjects would be significantly higher after the intervention than before. Although the mean FS score of the meditation subjects was higher after the intervention than before, this difference was not statistically significant ($p=0.56$; see Table 3, Figure 3D). Mean FS scores at the beginning ($p=0.74$) and end of the study period ($p=0.82$) were not statistically different between meditation and control subjects (See Figure 3B). Although there seemed to be a robust correlation between helpfulness rating and change in FS score in meditation subjects ($r=0.51$; see Figure 4D), this was not statistically significant ($p=0.11$). There was no significant correlation between compliance score and change in FS score over time ($p=0.95$; see Figure 4B).

Since there was incomplete data for some subjects, data for the two-week time point (time 2) was analyzed separately based on all subjects with complete data for both time 1 and time 2 for each of the parameters measured (See Appendix F). After two weeks, the mean PSS score for those subjects decreased significantly in the meditation subjects ($\Delta PSS = -6.30 \pm 1.39$, $n=10$, $p=0.001$) but there was no significant change in the controls ($\Delta PSS = +0.90 \pm 1.16$, $n=10$, $p=0.46$). However, there was no significant change after two weeks in mean FS score for either meditation ($\Delta FS = 1.50 \pm 1.67$, $n=10$, $p=0.39$) or control subjects ($\Delta FS = -1.90 \pm 0.43$, $n=10$, $p=0.20$).

Table 3: Mean Perceived Stress, Flourishing, and RTL Values Pre- and Post-Intervention

Mean Perceived Stress, Flourishing, and RTL Values Pre- and Post-Intervention[c]			
Changes in Mean PSS Scores	**Control Mean (n=10)**	**Meditation Mean (n=11)**	**p-value[b]**
PSS 1	15.5 (± 1.35)	20.0 (± 2.18)	0.10
PSS 3	17.0 (± 1.41)	14.9 (± 1.75)	0.37
Δ PSS	1.50 (± 0.86)	-5.09 (± 1.98)	0.01
p-value[a]	0.12	0.03	
Changes in Mean FS Scores	**Control Mean (n=10)**	**Meditation Mean (n=12)**	**p-value[b]**
FS 1	45.4 (± 2.73)	44.08 (± 2.49)	0.74
FS 3	43.9 (± 0.95)	44.83 (± 0.86)	0.82
Δ FS	-1.50 (± 3.01)	0.75 (± 2.75)	0.18
p-value[a]	0.15	0.56	
Changes in Mean RTL Values	**Control Mean (n=6)**	**Meditation Mean (n=9)**	**p-value[b]**
RTL 1	2.34 (±0.40)	2.30 (± 0.33)	0.94
RTL 3	2.25 (± 0.40)	2.80 (± 0.33)	0.29
Δ RTL	-0.095 (± 0.28)	0.21 (± 0.23)	0.35
p-value[a]	0.75	0.27	

[a]two-tailed p-value for change between time points, paired t-test
[b]two-tailed p-value for difference in average change between meditation and control subjects, unpaired t-test
[c]different sets of subjects for each analysis depending on availability of data. For PSS score data at times 1 and 3 there were 10 control and 11 meditation subjects with full data. For FS score, all subjects had available data, and for RTL values, there were 6 control and 9 meditation subjects with full data. For RTL values, subject samples were eliminated due to unreliable data points below threshold for detection in the qPCR reaction.

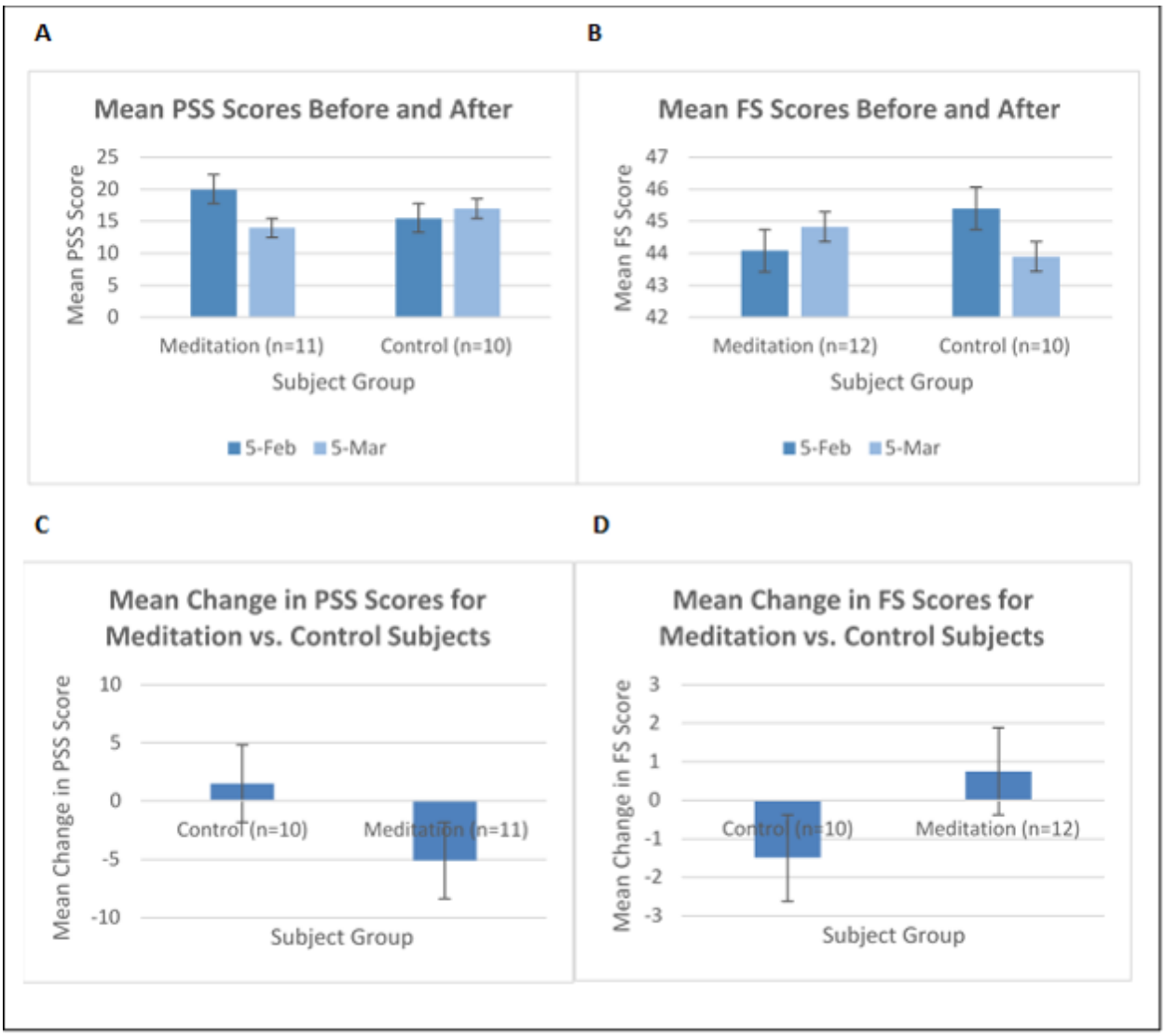


Figure 3 *(A) Mean PSS score in meditation and control subjects before and after is shown. There was a significant decrease in PSS score of meditation subjects (n=11, p=0.03) but no significant change in that of the controls (n=10, p=0.12). (B) Mean FS score in meditation and control subjects before and after is shown, and there was no significant change in mean FS score in meditation (n=12, p=0.56) or control (n=10, p=0.15) subjects. (C) Mean change in PSS score in meditation subjects and controls, with PSS score of meditation subjects changing significantly more than that of controls (p=0.01). (D) Mean change in FS score in meditation and control subjects, with no significant difference between amount of change in both groups (p=0.18).*

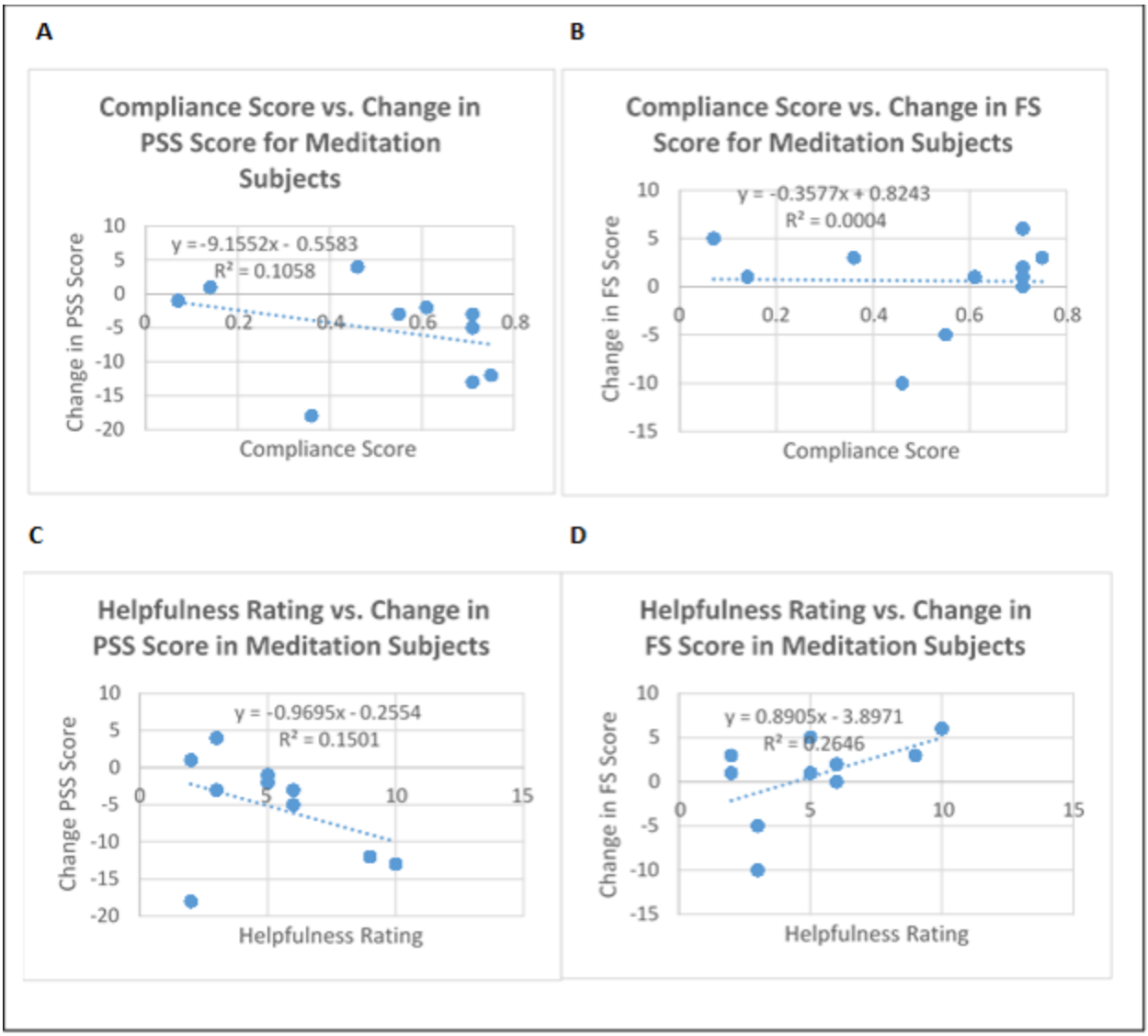


Figure 4 *(A) Correlation between change in PSS score and compliance score is shown for all subjects with available data for both parameters. This was not statistically significant (n=10, r=0.33, p=0.36). (B) Correlation between change in FS score and compliance score for all subjects with available data. This was also not statistically significant (n=11, r=0.02, p=0.95). (C) Correlation between change in PSS score and helpfulness rating, not statistically significant (n=10, r=0.39, p=0.27). (D) Correlation between change in FS score and helpfulness rating, not statistically significant (n=11, r=0.51, p=0.11). All statistical analyses were done with linear regression and Pearson's test.*

Relative Telomere Length

The main parameter of interest in this research was the effect of the meditation intervention on telomere length in subjects. The hypothesis was that telomeres would shorten less on average in meditation subjects than in controls. The results were interesting, in that mean

relative telomere length (RTL) increased in meditation subjects after the intervention (Figure 5B). However, this change was not statistically significant ($p=0.27$; see Table 3). The differences in mean RTL between meditation and control subjects were not significant before ($p=0.94$) or after ($p=0.29$) the intervention (see Table 3, Figure 5A). While the change in mean RTL after the study period was greater in meditation subjects than controls (see Figure 5B), this difference in amount of change was not statistically significant ($p=0.35$). The compliance scores and helpfulness ratings of the meditation subjects seemed to be positively associated with changes in RTL ($r=0.59$ and $r=0.35$, respectively; see Figure 5C and 5D), but these correlations were not statistically significant ($p=0.12$ and $p=0.41$, respectively).

Over the first two weeks of the intervention, surprisingly in the subjects studied, mean relative telomere length values increased in both controls ($\Delta RTL= +0.60 \pm 0.55$, $n=9$, $p=0.31$) and meditation subjects ($\Delta RTL= +1.59 \pm 1.81$, $n=10$, $p=0.40$), but more so in meditation subjects (See Appendix F). However, these changes were not statistically significant in control or meditation subjects, and the mean change in RTL value was not significantly greater in meditation subjects than controls ($p=0.62$).

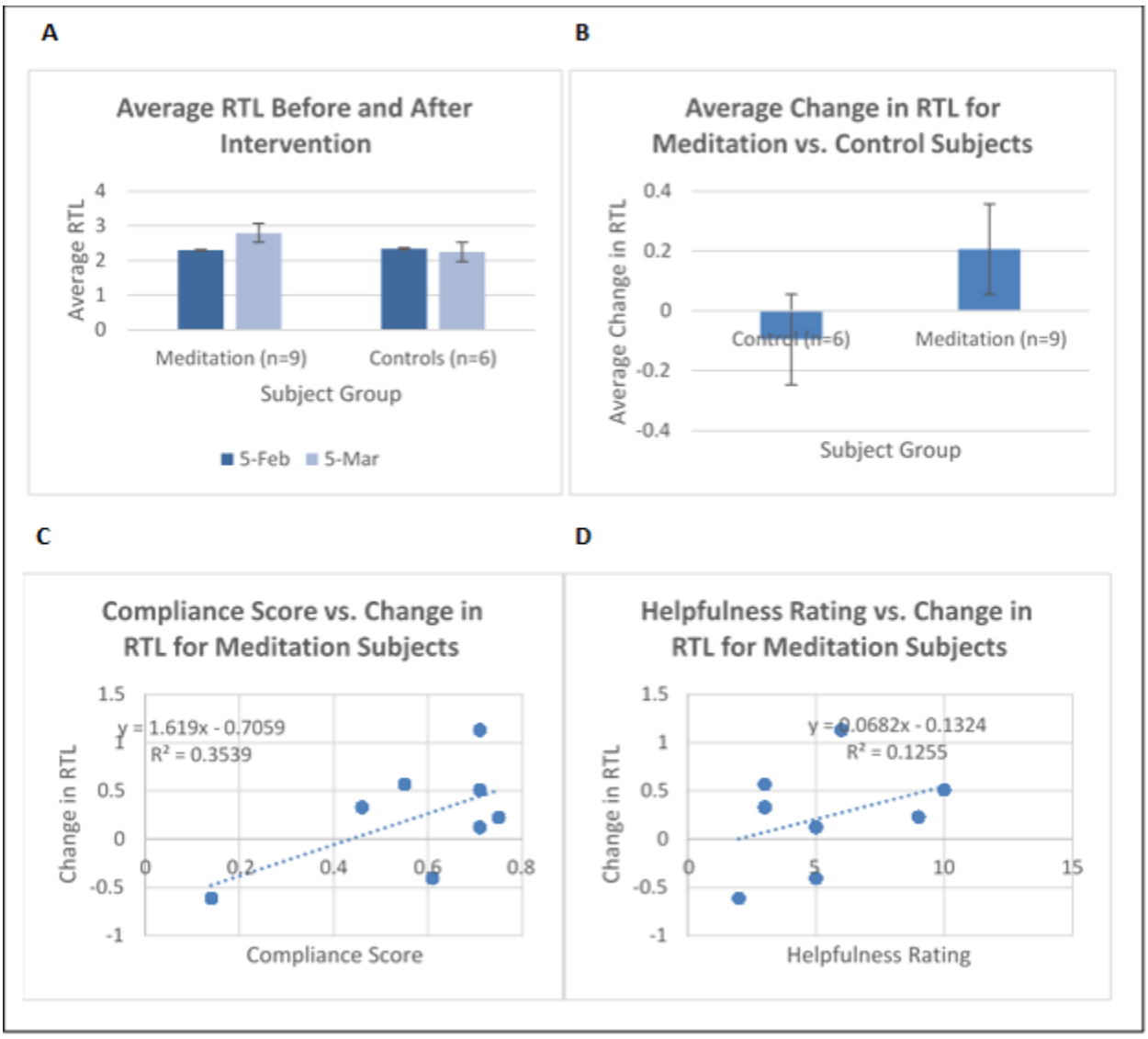


Figure 5 *(A) Change in average RTL value after the intervention was not statistically significant in meditation (n=9, p=0.27) or control (n=6, p=0.75) subjects. (B) Change in average RTL value was not significantly different between meditation and control subjects (p=0.35). (C) Compliance score was correlated with change in RTL, however this was not significant (n=8, r=0.59, p=0.12). (D). Helpfulness rating was also correlated with change in RTL, but not statistically significant (n=8, r=0.35, p=0.34).*

Relative Telomere Length and Psychological Measures

The main hypotheses of the present study were based on the assumption that there is a correlation between changes in psychological parameters and changes in relative telomere length. It was hypothesized that changes in relative telomere length over the four-week period

would be inversely correlated with changes in perceived stress and directly associated with changes in psychological well-being. It was also hypothesized that changes in perceived stress and psychological well-being would be inversely related. For these correlational analyses, data points for all subjects were used, regardless of group, in order to allow for a greater level of overall statistical strength. As expected, there was a significant inverse correlation between changes in PSS scores and changes in FS scores ($p=0.04$, $r=0.46$; see Figure 6A).

While there seemed to be a weak inverse correlation between changes in PSS scores and changes in relative telomere length (RTL) values ($r=0.23$; see Figure 6B), this was not statistically significant ($p=0.42$). Where was a weak direct correlation between changes in FS scores and changes in RTL values ($r=0.18$), this was also not statistically significant ($p=0.52$).

The same analysis was done using only meditation subjects, but there was no statistically significant correlation between change in PSS score and change in FS score ($n=11$, $r=0.57$, $p=0.07$), between change in PSS score and change in RTL value ($n=8$, $r=0.26$, $p=0.54$), or between change in FS score and change in RTL value ($n=9$, $r=0.06$, and $p=0.87$). However, the lack of statistical significance may be due to lack of a sufficient sample size.

In order to see if there was a correlation between psychological parameters and relative telomere length at a given point in time, this was measured in all subjects using data points from all times measured. Overall, higher PSS scores seem to be inversely negatively correlated with FS scores at any given time ($n=58$, $r=0.27$, $p=0.04$), but there is no significant correlation between cross-sectional PSS scores and RTL values ($n=55$, $r=0.02$, $p=0.90$) or between cross-sectional FS scores and RTL values ($n=56$, $r=0.02$, $p=0.88$).

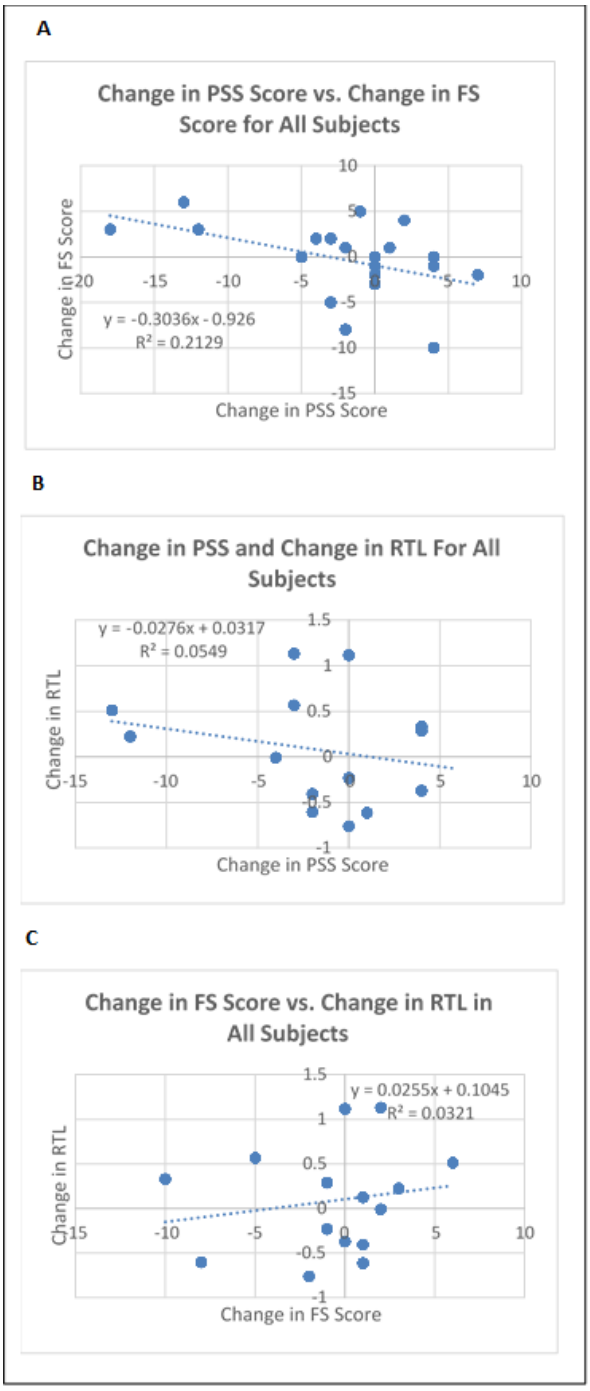


Figure 6 *(A) The correlation between change in PSS score and change in FS score for all subjects with data points for times 1 and 3 for both PSS and FS. The correlation between change in PSS score and change in FS score is statistically significant (n=20, r=0.46, p=0.04). (B) The correlation between change in PSS score and change in RTL value is shown for all subjects. There is no significant relationship between the two (n=14, r=0.23, p=0.12). (C) The correlation between change in FS score and change in RTL value is shown for all subjects, not statistically significant (n=15, r=0.18, p=0.52).*

Discussion

The results of this study partially support the hypotheses and provide ground for future, more large-scale studies on meditation and telomere length in college students. There was a significant decrease in levels of perceived stress in meditation subjects after two weeks of the intervention, and the decrease in stress remained significant after one month (Table 3). The amount of decrease in perceived stress after one month was correlated with compliance scores and with participants' subjective ratings of the helpfulness of the meditation class in reducing their stress levels (Figures 4A and 4C), which supports the notion that these results may be due to the intervention. This supports the hypothesis that meditation, even in the short term, decreases psychological stress. Perhaps, as the participants learned mindfulness and attempted to incorporate mindful thinking into their daily lives, they may have been able to decrease the frequency of threat appraisals, rumination, and emotional distress.

In contrast, levels of psychological well-being, as measured by the Flourishing Scale (FS), did not change significantly after the intervention. The Flourishing scale measures levels of subjective self-esteem, sense of purpose in life, level of optimism, and perceived success in relationships (Diener et al., 2010), and perhaps these cognitions take longer than one month to change substantially. However, notably most participants in the meditation group did have increased FS scores, and there was a positive correlation between FS score and the participants' ratings of helpfulness of the intervention in reducing stress (Figure 4D). This suggests that, while not significant, the intervention may have had an effect on positive psychological well-being in some participants.

Mean telomere length also did not change significantly for participants in the meditation class at the end of the one-month study, which suggests that a longer period of time may be

required before significant changes are noted. However, meditation class participants had more telomere elongation than control subjects after two weeks and four weeks (Table 3, Figures 5A and 5B), even though the mean telomere length of control subjects also increased after two weeks. Telomere elongation was also positively correlated with compliance to the intervention ($r= 0.59$) and, to a lesser extent, to participants' helpfulness ratings ($r= 0.35$) (Figures 5C and 5D). This provides evidence that meditation may have a positive effect on telomere elongation, but this needs to be supported by more larger-scale studies.

There was a significant inverse correlation between changes in psychological stress and changes psychological well-being ($p= 0.04$, see Figure 6A) as measured by the Perceived Stress Scale and the Flourishing Scale, which supports the notion that decreases in stress may be linked with increased levels of positive cognition and vice versa. However, there were no significant correlations between changes in perceived stress and changes in telomere length or between changes in psychological well-being and changes in telomere length. While there seems to be a weak negative relationship between increased stress and telomere elongation, and a weak positive relationship between increased psychological well-being and telomere elongation, this needs to be supported by further studies before any conclusions can be reached. If this is supported by more substantial evidence, it would support the hypothesis that short-term changes in psychological health may have an effect on telomere length homeostasis.

The results of this study provide the basis for questions that may need to be addressed by the scientific community in order to better understand telomere length dynamics. While usually telomeres are known to shorten over the years, evidence suggests that telomere length may fluctuate in the short term (Epel, 2012). One study on telomere length in multiple tissues including buccal cells showed that after 2-6 years, around 15-25% of individuals had longer

telomeres (Epel, 2012). In the present study, even some of the control subjects as a group had an increased mean telomere length after two weeks. However, after one month of the study, their mean telomere length was actually slightly lower than at the beginning of the study. This kind of telomere length dynamic may be explained by a model of telomere equilibrium under stress (Epel, 2012). This model suggests that stress leads to increased cell turnover, possibly due to necrosis or apoptosis, and that these new cells have longer telomeres but shorten rapidly due to elevated rates of cell division, to compensate for damaged cells (Epel, 2012). This could be a possible explanation for the fluctuations in telomere length observed in the control subjects.

In meditation subjects, by contrast, mean telomere length was consistently higher after two and four weeks of the intervention than it was at onset. Perhaps the mechanisms of telomere lengthening in the meditation subjects were based more on increased cell-specific activation of telomerase rather than cell turnover and influx of new cells with longer telomeres. It may have been helpful to have studied telomerase activity to study any associations between changes in telomere length and telomerase activity in meditation subjects to better elucidate this. We propose various possible mechanisms through which decreased psychological stress and improved psychological well-being could lead to telomerase-mediated telomere elongation. Since expression of the Tert subunit of telomerase is the main limiting factor in telomerase activity (Shawi et al., 2008), factors regulating its expression may be of interest. One possibility is that decreases in psychological stress led to decreased levels of inflammatory cytokines, some of which are thought to down-regulate the transcription of the Tert subunit (Lin et al., 2012; Houben et al., 2008). This would allow for increased telomerase expression and subsequent elongation. Psychological well-being is associated with increased levels of anabolic hormones, which are thought to up-regulate telomerase expression (Epel et al., 2009), and if meditation

improved well-being, this could also be a potential mechanism. It may be that anabolic hormones also up-regulate tankyrase, which is a protein involved in opening the T-loop to allow for telomerase-mediated elongation (Houben et al., 2008). It could also be that oxidative damage at the transcriptional level could simply down-regulate telomerase expression, or make it more difficult for telomerase bind to the telomere to elongate it. Thus, decreased levels of oxidative stress could reverse or prevent those mechanisms.

One issue to consider is that this study focused on telomere length in buccal cells. Many cells need constant replacement and so get replenished by stem cells on a regular basis (Houben et al., 2008), and this may be the case for buccal cells. Naïve cells tend to have longer telomeres (Epel, 2012). Moreover, there may have been immune cells that infiltrated in the mouth cavity with saliva in some subjects, possibly due to poor oral hygiene or infection (Shalev, 2012). Since cell length may vary between tissue types (Shalev, 2012), this could have affected the results. Buccal cells are somatic cells, and so may have limited or no telomerase expression in the first place. Thus, it may be important to have a better understanding of telomerase expression and cellular turnover rates in buccal cells to better elucidate the mechanisms involved. However, studies do show that measurements of telomere length in different cell types within an individual are correlated, and other studies have found that buccal cells are predictive of risk for certain diseases, hence support for using buccal cells in the present study (Houben et al., 2008).

It is important to note that biological factors other than stress could have an important effect on short-term telomere length dynamics. Studies show that telomerase preferentially targets shorter telomeres (Epel, 2012), perhaps as a homeostatic mechanism. This suggests that shorter initial telomere length may be linked to a greater amount of elongation over time, at least in short term studies. We did a quick test of this using linear regression, and found that there was

actually a very statistically significant positive correlation between initial telomere length and increase in telomere length after two weeks ($p < 0.001$). Thus, individuals with longer telomeres initially tended to have telomere elongation after two weeks, and those with shorter initial telomeres had no change or telomere shortening after that same time period. The same analysis was done for the next two weeks, but there was no significant correlation. When the relationship between initial telomere length and total change in telomere length after the study was analyzed, there seemed to be an inverse correlation as expected, but this was not statistically significant. These results are equivocal and suggest the need for larger studies on short term changes in telomere length.

Overall, this study elucidates some new findings on the potential effects of a mindfulness meditation intervention on short-term changes in telomere length in college students, but more importantly it urges the need for further research on the complex pathways involved in the link between psychological health and telomere biology. Those findings may translate towards a better understanding of preventive medicine, health, and age-related disease.

Appendix A: DNA Extraction Concentrations and Metrics

Subject ID	Collection Time 1			Collection Time 2			Collection Time 3		
	ng/µL	**260/280**	**260/230**	**ng/µL**	**260/280**	**260/230**	**ng/µL**	**260/280**	**260/230**
C1	**267.0**	1.65	1.26	**202.0**	1.93	1.23	**87.4**	1.61	0.57
C2	**863.4**	1.63	1.30	**223.5**	1.84	1.07	**79.6**	1.91	0.95
C3	**48.9**	1.67	0.63	**48.4**	1.95	0.80	**69.8**	1.90	0.66
C4	**32.1**	1.64	0.53	**106.9**	1.92	1.10	**57.5**	1.62	0.47
C5	**68.4**	1.76	0.67	**53.7**	1.74	0.50	**29.7**	152	0.50
C6	**46.9**	1.77	0.60	**32.1**	1.61	0.55	**85.7**	1.88	0.97
C7	**100.5**	1.71	1.08	**91.0**	1.95	1.13	**34.3**	1.99	0.67
C8	**63.2**	1.65	0.84	**37.3**	1.82	0.65	**14.3**	1.74	0.33
C9	**41.5**	1.56	0.61	**33.2**	1.81	0.59	**15.8**	1.82	0.35
C10	**36.0**	1.71	0.31	**26.6**	1.90	0.51	**147.3**	1.92	1.28
M11	**307.0**	1.48	1.24	**26.9**	1.92	0.49	**43.4**	1.74	0.33
M12	**47.3**	1.66	0.56	**27.7**	1.86	0.53	**-**	-	-
M13	**50.4**	1.54	0.72	**29.0**	1.85	0.60	**54.3**	1.89	0.86
M14	**57.8**	1.60	0.76	**42.4**	1.97	0.73	**26.9**	1.87	0.58
M17	**111.7**	1.93	0.45	**70.9**	1.91	0.73	**116.2**	1.97	1.22
M18	**47.1**	1.62	0.63	**27.1**	1.76	0.48	**52.2**	1.96	0.87
M23	**60.5**	1.85	0.39	**15.1**	1.87	0.35	**52.2**	1.96	0.87
M24	**33.3**	1.68	0.34	**60.3**	1.98	0.83	**39.3**	1.71	0.41
M26	**211.5**	1.32	1.15	**-**	-	-	**68.1**	1.78	0.88
M27	**234.5**	1.47	1.07	**66.0**	1.84	0.84	**79.9**	1.90	1.53
M28	**258.0**	1.47	1.20	**13.5**	1.71	0.31	**294.6**	1.91	1.53

Appendix B: Perceived Stress Scale (Cohen et. al, 1983)

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.

Name _____ Date _____

Age _____ Gender (Circle): M F Other _____

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset
because of something that happened unexpectedly?..... 0 1 2 3 4
2. In the last month, how often have you felt that you were unable
to control the important things in your life? 0 1 2 3 4
3. In the last month, how often have you felt nervous and "stressed"? 0 1 2 3 4
4. In the last month, how often have you felt confident about your ability
to handle your personal problems? 0 1 2 3 4
5. In the last month, how often have you felt that things
were going your way?..... 0 1 2 3 4
6. In the last month, how often have you found that you could not cope
with all the things that you had to do? 0 1 2 3 4
7. In the last month, how often have you been able
to control irritations in your life?..... 0 1 2 3 4
8. In the last month, how often have you felt that you were on top of things?.. 0 1 2 3 4
9. In the last month, how often have you been angered
because of things that were outside of your control?..... 0 1 2 3 4
10. In the last month, how often have you felt difficulties
were piling up so high that you could not overcome them? 0 1 2 3 4

Appendix C: Flourishing Scale (Diener et al, 2010)

Below are 8 statements with which you may agree or disagree. Using the 1–7 scale below, indicate your agreement with each item by indicating that response for each statement.

• 7 - Strongly agree

• 6 - Agree

• 5 - Slightly agree

• 4 - Neither agree nor disagree

• 3 - Slightly disagree

• 2 - Disagree

• 1 - Strongly disagree

____ I lead a purposeful and meaningful life

____ My social relationships are supportive and rewarding

____ I am engaged and interested in my daily activities

____ I actively contribute to the happiness and well-being of others

____ I am competent and capable in the activities that are important to me

____ I am a good person and live a good life

____ I am optimistic about my future

____ People respect me

Appendix D: Post-Completion Questionnaire Items of Interest

1) **All Subjects.** During the period of the study, did you undergo any stressful life situations such as the death of a loved one, or a difficult personal situation? (Please circle one)

a) No

b) Yes

If so, please rate how much emotional stress this situation caused you (1-10): _____

Would you perceive the situation as more as a challenge or more as a threat? _____

2) **Intervention Subjects Only.** How much do you feel like you complied with the intervention in terms of daily 20 minute meditation practice? (Please circle one)

a) Practiced meditation on my own for 20 minutes every day or almost every day over the course of the month.

b) Practiced meditation for 20 minutes on average around 3-5 days per week.

c) Practiced meditation for 20 minutes on average around 1-2 days per week.

d) Practiced meditation infrequently on my own (less than once a week on average).

3) **Intervention Subjects Only.** Do you feel as if this class helped you cope with stress? 1 = not at all, 10 = very much so

Rating (1-10): _____

Appendix E: Raw Data for Attendance and Post-Completion Questionnaire

Class Session	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Total Attendance (out of 12 students)	10	7	6	8	6	3	3	2

Life Stress	**Meditation (n=11)**	**Control (n=8)**
# Students w/ Life Stress	3	4
Mean Rating	9.3	6.2
# Challenging	10	7
# Threatening	1	1

This table is based on a total of 19 responses received from the post-completion questionnaire. Students were asked whether they experienced a stressful life situation during the time of the study. An approximately equal amount of participants in each group experienced a stressful life event, and only one subject in each group described their situation as being threatening. However, the average rating of emotional stress induced by the situation was significantly higher in meditation than control subjects (p=0.01).

Appendix F: Changes in Mean PSS Scores, FS Scores, and RTL Values at Baseline and 2 Weeks***

Changes in Mean PSS Scores at Baseline and 2 Weeks	Control (n=10)	Meditation (n=10)	p-value**
PSS 1	15.50 (± 1.35)	20.10 (± 2.41)	0.11
PSS 2	16.40 (± 1.42)	13.80 (± 2.09)	0.32
ΔPSS	0.9 (± 1.16)	-6.3 (± 1.39)	0.002
p-value*	0.46	0.001	

Changes in Mean FS Scores at Baseline and 2 Weeks	Control (n=10)	Meditation (n=10)	p-value**
FS 1	45.40 (± 2.73)	45.60 (± 3.09)	0.96
FS 2	43.50 (± 2.76)	47.10 (± 2.14)	0.32
ΔFS	-1.90 (± 0.43)	1.50 (± 1.67)	0.13
p-value*	0.20	0.39	

Changes in Mean RTL Values at Baseline and 2 Weeks	Control (n=9)	Meditation (n=10)	p-value**
RTL 1	2.35 (± 0.34)	2.26 (± 0.28)	0.84
RTL 2	2.95 (± 0.45)	3.86 (± 0.73)	0.64
ΔRTL	0.60 (± 0.55)	1.59 (± 1.81)	0.62
p-value*	0.31	0.40	

*two-tailed p-value for change between time points, paired t-test

**two-tailed p-value for difference in average change between meditation and control subjects, unpaired t-test

***different sets of subjects for each analysis depending on availability of data

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