

# Three months-longitudinal changes in relative telomere length, blood chemistries, and self-report questionnaires in meditation practitioners compared to novice individuals during midlife

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## Abstract

Aging accelerates during midlife. Researches have shown the health benefits of mind-body intervention (MBI). However, whether MBI is involved with aging process has not been well understood. In this study, we approach to examine the relations of MBI with this process by investigating an aging marker of the peripheral blood, blood chemistry, and self-report questionnaires. A quasi-experimental design was applied. Experienced MBI practitioners participated in a 3-month intensive meditation training, while the age, gender-matched MBI-naïve controls led a normal daily life. Measurements were taken at before and after the 3 months for relative telomere length (RTL), blood chemistry, and self-report questionnaires including items about sleep quality, somatic symptoms, depression, anxiety, stress, emotional intelligence (EI), and self-regulation. For RTL, the repeated measures analysis of variance showed a significant group\*time interaction ( $P = .013$ ) with a significant post hoc result ( $P = .030$ ) within the control group: RTL was significantly reduced in the control while it was maintained in the meditation group. In repeated measures analysis of variance for blood chemistries, there were significant group differences between the groups in glucose and total protein. In the post hoc comparison analysis, at post measurements, the meditation group exhibited significantly lower values than the control group in both glucose and total protein. There were significant group-wise differences in the correlations of RTL with triglyceride (TG), high-density lipoprotein (HDL), glutamic oxaloacetic transaminase and glutamic pyruvic transaminase. Any of self-report results did not show significant changes in group\*time interaction. However, there were group differences with significant ( $P < .05$ ) or a tendency ( $.05 < P < .1$ ) level. There were significant improvements in depression, stress and EI as well as tendencies of improvement in sleep quality and anxiety, in the meditation group compared to the control group. Our results suggest that meditation practice may have a potential to modify aging process in molecular cellular level combined with changes in psychological dimension.

**Abbreviations:** ANOVA = analysis of variance, EI = emotional intelligence, GLU = glucose, GOT = glutamic oxaloacetic transaminase, GPT = glutamic pyruvic transaminase, HDL = high-density lipoprotein, HPA = hypothalamus-pituitary-adrenal, LDL = low-density lipoprotein, MBI = mind-body intervention, RTL = relative telomere length, TC = total cholesterol, TG = triglyceride, TP = total protein.

**Keywords:** aging, blood chemistry, meditation, midlife, mind-body intervention, telomere length

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The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files]

Data is contained within the article or supplementary material.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of University of Brain Education (IRB201809-01) and registered in Clinical Research Information Service (KCT0004965).

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## 1. Introduction

Mind-Body Interventions (MBIs) such as meditation, yoga, and qigong alter neural systems related with stress regulation, inducing changes in the body system throughout the hypothalamus-pituitary-adrenal (HPA) axis, autonomic nervous system, immune system, and endocrine systems.<sup>[1]</sup> As MBI impacts multiple body networks, consistent MBI practice can elicit various psychological and physiological changes in the body. Accumulated research has shown that various types of MBI improves psychological conditions (such as depression, anxiety, and stress) and related body symptoms (such as pain, sleep).<sup>[2–4]</sup> Recent findings have suggested that psychological distress is related with the aging process<sup>[5]</sup> and MBI, which can alleviate psychological issues, may delay the aging process especially in midlife,<sup>[6–8]</sup> but not in younger populations.<sup>[9,10]</sup> Among biological age markers, telomere length is recognized as representative of cellular aging.<sup>[11]</sup> Telomeres are short repeating sequences found at the end of chromosomes that are involved in the preservation of chromosomal integrity. Telomere length has been found to decrease due to aging, stress, or disease.<sup>[12–14]</sup>

Interestingly, telomere length has been shown to be affected by MBI practice. In one study (mean age 51 years old), a 1-month intensive insight meditation increased the telomere length of long-term meditators compared to the matched meditation-experienced people who did not perform the intensive training.<sup>[17]</sup> Moreover, in breast cancer survivors of mean age 54, telomere length was found to be maintained by psychosocial interventions including mindfulness-based cancer recovery and supportive-expressive group therapy, while it was reduced in the control group.<sup>[8]</sup> On the other hand, a meditation training in younger meditation-novice adults (mindfulness-based stress reduction for adults of mean age 27<sup>[9]</sup>; or mental training cultivating interoception, attention, compassion, perspective taking for adults of mean age 40<sup>[10]</sup>) did not induce a significant changes in telomere length after 8 weeks or 9 months, respectively. In summary, telomere length seems to be reduced even within a few months in susceptible groups such as midlife individuals or breast cancer survivors but not in younger populations, while stress-reduction techniques such as meditation seem to exhibit preventative effects against it.

MBI has also exhibited beneficial effects on glucose and lipid metabolism.<sup>[15,16]</sup> Telomere length has been reported as an independent risk factor of cardiovascular disease.<sup>[17]</sup> Besides, telomere length has been known to be associated with risk factors of cardiovascular diseases such as triglyceride (TG), total cholesterol (TC), and lipoproteins. In Taiwan's national health study for 3806 women, telomere length was highly reversely correlated with high TGs in fully adjusted models.<sup>[18]</sup> In US National health study for 6468 participants, telomere length was positively associated with high-density lipoprotein (HDL).<sup>[19]</sup> In addition, telomere length was inversely-correlated with TG and TC in men with type 2 diabetes mellitus<sup>[20]</sup> as well as healthy adults.<sup>[21]</sup> However, it is not well known whether the relation between telomere length and blood chemistry is affected by MBI.

Here, we investigate the effects of a specific type of MBI, which is called *Brain Education Meditation*, on biological age, blood chemistries, and psychological features in midlife. *Brain Education Meditation*<sup>[22]</sup> is the same meditation training previously known as *brain wave vibration*<sup>[23–26]</sup> or *brain respiration training*,<sup>[27,28]</sup> with minor modifications. It is a modernized form of traditional Korean Sundo meditation and has been reported on its effects on mood,<sup>[26,29,30]</sup> sleep,<sup>[23,24]</sup> brain structure and activities<sup>[25,31,32]</sup> as well as inflammatory states,<sup>[22,33]</sup> blood catecholamine level,<sup>[29,30]</sup> nitric oxide level,<sup>[26]</sup> and lipid level.<sup>[22]</sup> In this study, meditators who have experienced *Brain Education Meditation* and age, sex and education-matched meditation naïve individuals were recruited and the following factors were measured twice at baseline and after 3 months of intensive training (for experimental group) or daily life (for control group):

relative telomere length (RTL); blood chemistries (glucose [GLU], TC, low-density lipoprotein [LDL], TG, HDL, total protein [TP], glutamic oxaloacetic transaminase [GOT], glutamic pyruvic transaminase [GPT], lactate dehydrogenase, Blood Urea Nitrogen, creatinine); self-report questionnaires on sleep quality, somatic symptoms, depression, anxiety, stress, emotional intelligence (EI), and self-regulation. We attempt to uncover correlations between these factors and provide support for the efficacy of the intervention as a training regime to improve the health of midlife individuals.

## 2. Methods

### 2.1. Participants

This study was conducted as part of a project to understand the psychological and physiological effects of *Brain Education Meditation* between September and December 2018 at 3 research institutions located in Seoul and Cheonan, Republic of Korea. It was designed as a quasi-experimental study design with long term meditators and age, gender, education, religious state-matched meditation-naïve controls, due to difficulties in recruiting enough novice participants for a randomized controlled trial. For the experimental group, participants were recruited at the training centers in Seoul, Daejeon, and Pusan, Republic of Korea. Control participants without meditation experience were recruited through social network service, flyers, bulletin boards, and open lecture presentation promotion at the same institutions. Healthy adults participated in the study, excluding those who had difficulties in listening, writing, or understanding, or restrictions in physical activities performed in the training group. In the control group, those who regularly train with meditation for last 3 months were excluded. The applicants were all Korean. Those who were in disease progression (e.g., cancer,<sup>[34]</sup> chronic inflammation,<sup>[35]</sup> coronary artery disease<sup>[36]</sup>) or on medication which could affect telomere length were excluded. All participants were given training service coupons worth 100,000 won after all measurements. The research was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All the participants provided written informed consent. The research protocol was approved by the Ethics Committee of the University of Brain Education and registered in Clinical Research Information Service (KCT0004965). In this study, only the data of participants whose blood sample was qualified for RTL analysis were analyzed. For RTL analysis, during the process of sample preservation, DNA extraction and quantitative PCR for telomere measurement, individuals were dropped if there was a loss of either pre or post sample, sample preservation issues, low DNA concentration, the integrity of genomic DNA of pre or post measurement, or repeatability issues. Eventually the blood samples of 38 individuals (21 in the experimental group and 17 in the control group) were used for the final analysis. Among them, due to the omission of answers to questions for either the pre- or post-questionnaires, 3 individuals were dropped for self-report analysis. In this study, data of blood chemistry as well as self-report analyses are only limited to the individuals whose samples were qualified for the telomere length analysis (Table 1).

### 2.2. Intervention

The experimental group participated in the meditation training program for 90 minutes per day (2 group sessions and 5 home sessions per week) over 3 months under the guidance of professional instructors. The control group lived their regular daily lives during the same period without meditation-related training. One session consists of both meditative movements for body awareness and a focused attention meditation as follows.

**Table 1**  
**Subject demographics.**

Demographic characteristics	EXP	CTL	<i>P</i> value ( <i>t</i> test or $\chi^2$ )
Number of subjects	21	17	-
Age (yrs)	48.33 $\pm$ 1.89	51.18 $\pm$ 1.99	.310
Gender (Female/Male)	19/ 2	13/ 4	.239
Education period (yrs)	15.33 $\pm$ 0.77	14.29 $\pm$ 1.14	.443
Occupation (hired/not hired)	21/ 0	14/ 3	.045*
Religious status (religious/ non-religious)	13/ 8	8/ 9	.360
Duration of practice (yrs)	10.33 $\pm$ 1.11	-	-
Practice time per day (min)	42.14 $\pm$ 6.43	-	-
Frequency of practice per day	2.52 $\pm$ 0.44	-	-

The values indicate mean  $\pm$  standard error or number of subjects. *P* values represent the values of the Student's *t*-test or Chi-square test.

CTL = control, EXP = experiment.

\* $P \leq .05$ .

### Step 1. Meditative Movement I<sup>[23,24]</sup>

- Warm-Up (10 minute): Mild whole-body exercise with meditative lines to increase body awareness.

- *Brain wave vibration* (10 minute): A sitting or standing moving meditation with gentle rhythmic shakes of the neck and body, sometimes combined with gentle abdominal tapping.

- Jigam (5 minute): Calming the mind and being still by focusing on the sensation of one's hands.

- Breathing postures (10 minute): Calming the mind and being still by focusing on one's breath, combined with several body postures.

- Dahnmu (5 minute): Energy dance.

- Warm-Down (5 minute): Mild whole-body exercise with meditative lines for warm-down.

Step 2. Meditative Movement II (10 minute): One among twelve different postures for improving muscle strength and attention depending on the level of each participant.

Step 3. *Sangdahnjeon* Meditation (30 minute): Focused attention meditation (breath, body sensation, imagery).

Step 4. Wrap-Up (5 minute): Share one's feelings and experiences through the session.

### 2.3. Blood chemistry and RTL measurement

Blood collection took place before and after the intervention. Participants were required to fast for at least 8 hours before blood collection, as diet-dependent factors such as blood glucose were measured from the collected blood. Blood samples for blood chemistry as well as other analysis, which are not included in this study, were collected in a 10 ml EDTA tube and immediately mixed. After centrifugation at  $1500 \times g$  for 10 min, the plasma was subjected to blood chemistry analysis (BS-200 Chemistry Analyzer, Mindray, China). Whole blood samples for RTL measurement were collected in a 3 ml EDTA tube, immediately mixed and stored at a -80 degree freezer at the University of Brain Education until analysis, and the related experiments were conducted in the lab of the same university. Frozen blood did not go through repeated freeze thaw cycles. It was melted only once for extraction, genomic DNA of peripheral blood cells was extracted by Gentra Puregene Blood Kit Plus (Cat No. 158489, Qiagen). The 260/280 ratio of extracted DNA was within the range of 1.7 - 2.0 (average 1.89), with an average concentration of 249.45 ng/ $\mu$ L. Each genomic DNA was adjusted to 100 ng/ $\mu$ L, and to minimize the freeze-thaw cycle, the genomic DNA samples were aliquoted and stored at -80 degrees with a stock concentration of 10 ng/ $\mu$ L. A total volume of 8  $\mu$ L containing 10 ng/

1  $\mu$ L genomic DNA 2  $\mu$ L, 10  $\mu$ M forward and reverse primer 1  $\mu$ L each (corresponding to telomere or 36BR), Power Up SYBR Green Master Mix (Life Technologies, Austin, TX) 4  $\mu$ L was used for 1 replicate of a 96 well plate in a quantitative PCR reaction. Real time PCR was performed in the Step One Plus Real-Time PCR System (Applied Biosystems), independently by 2 researchers who were kept blinded from any information related with the sample. Samples were run with triplicate technical repeats in a 96 well plate. Every run contained reference DNA which contained 28 randomly mixed genomic DNA samples to calculate inter-assay variability. The averaged inter- and intra-assay coefficient of variation of the data set was 6.69% and 1.34%, respectively. The measurement of RTL was adapted from Cawthon's reports<sup>[37,38]</sup> with minor modifications. The RTL was calculated by the ratio (T/S) of telomere product (T) to a single copy gene 36BR product (S) in the respective samples. Following primer sets were used for 36B4 (forward, 5'- CAGCAAGTGGGAAGGTGTAATCC-3'; reverse, 5'- CCCATTCTATCATCAACGGGTACAA-3') and telomere (forward, 5'- CGGTTTGTGTTGGGTTTGGGTT TGGGTTTGGGTTTGGGTT-3'; reverse, 5'- GGCTTGCCCTTACCCTTACCCT TACCCTTACCCTTACCCT-3').

## 2.4. Self-reports

Pittsburgh Sleep Quality Index is a self-report checking sleep quality and disturbances over a month and consists of 18 items.<sup>[39]</sup> Patient Health Questionnaire-15 is a questionnaire measuring the severity of somatic symptoms and consists of 15 items.<sup>[40]</sup> Depression Anxiety and Stress Scale-21 is a questionnaire which measures depression, anxiety and stress,<sup>[41]</sup> and consists of 21 items. EI scale designed for trait EI for Korean adults consisting of 20 items was used.<sup>[42]</sup> Goal-focused self-regulation scale measures goal orientation, positive thought, emotional regulation, and goal fulfillment and consists of 13 items.<sup>[43]</sup>

## 2.5. Statistics

An optimal total sample size of 34 participants required to perform a 2-way mixed-design analysis of variance (ANOVA) (effect size  $f = 0.25$  with a significance level set at  $\alpha = 0.05$ , power  $1 - \beta = 0.80$ ) was calculated using the G-Power software application. Statistical analyses were performed using SPSS, version 25. The  $\chi^2$  tests were used to investigate the group differences in the number of genders, jobs, and religious status of participants. The differences in age and education were tested using Student  $t$  tests. In line with prior research which showed the reduction of leukocyte telomere length along with age in adults,<sup>[44]</sup> age was included as a covariate in statistical models of RTL. Two-way mixed-design analysis of covariance with age as a covariate was used to investigate the effects of the group, treatment, and interaction between the group and treatment on the RTL. Two-way mixed-design ANOVA was used for blood chemistries and self-report results. A statistical significance was based on 2-sided statistical tests and was evaluated at a .05 level of significance.

### 3. Results

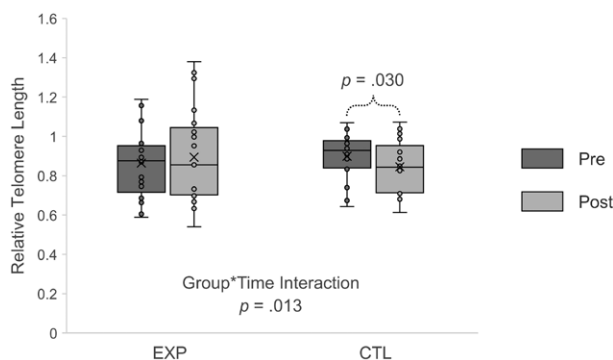
### 3.1. Changes in RTL

The age, gender, education period, occupation, and religious status were examined between the experimental and control groups (Table 1). The experimental and control groups ( $N = 21$  vs  $17$ ) were comparable in age ( $48.33 \pm 1.89$  vs  $51.18 \pm 1.99$  years old, mean  $\pm$  SE), gender ( $19/2$  vs  $13/4$ , female/male), education level ( $15.33 \pm 0.77$  vs  $14.29 \pm 1.14$  years, mean  $\pm$  SE), and religious status ( $13/8$  vs  $8/9$ , religious/non-religious). The employment rate showed a significant group difference ( $21/0$  vs  $14/3$ , hired/



not hired,  $P = .045$ ). The mean practice period, practice time per day, and frequency of practice per day of the meditation group were  $10.33 \pm 1.11$  years,  $42.14 \pm 6.43$  minutes, and  $2.52 \pm 0.44$  times, respectively. The control group had no prior meditation experience.

Next, we examined training-related changes in RTL with analysis of covariance (Table S1, <http://links.lww.com/MD/H452>, S2, <http://links.lww.com/MD/H453>, Fig. 1). There were no significant effects in group ( $P = .904$ ) or time ( $P = .189$ ) factors, but there was a significant effect in group by time interaction ( $P = .013$ , Table S1, <http://links.lww.com/MD/H452>, Fig. 1). post hoc Holm-Sidak comparisons indicated there were no significant differences in RTL between groups at the basal level ( $P = .512$ ), as well as post-treatment ( $P = .472$ , Table S2, <http://links.lww.com/MD/H453>). However, the control group exhibited a significant reduction in the RTL at the post-intervention compared to the pre-intervention period ( $P = .030$ ), while the experimental group showed a slight increase but not passing significance, in the RTL ( $P = .167$ , Table S2, <http://links.lww.com/MD/H453>, Fig. 1).



**Figure 1.** Changes in relative telomere length. Box & whisker plot on relative telomere length by group and time. 21/17 participants for EXP/CTL. Effect of interaction between group and time:  $P = .013$ . Post hoc Holm-Sidak comparison between CTL-pre and CTL-post:  $P = .030$ . Covariates appearing in the model is evaluated at the following value: age = 49.61. CTL = control, EXP = experiment, Pre = pre-measurement, Post = post-measurement.

### 3.2. Changes in blood chemistries

Results of blood chemistry analysis were analyzed by 2-way mixed-design ANOVA (Table 2). There were no significant changes in group\*time interaction, time or group differences in TC, TG, HDL, GOT, GPT, lactate dehydrogenase, Blood Urea Nitrogen, and creatinine. For LDL, there was a tendency of change in the interaction effect between group and time ( $P = .060$ ). In post hoc comparison, LDL was increased significantly at the post intervention compared to the pre-intervention period in the control group ( $P = .029$ ), while little change was observed in the EXP group ( $P = .745$ ). The EXP group exhibited lower levels of GLU or TP compared to the control group ( $P = .034$  and  $.015$  respectively). In both GLU and TP, the group difference was more prominent at post than pre-intervention ( $P = .042$  and  $.014$  respectively), while they exhibited slight changes at pre-intervention but without passing significance ( $P = .072$  and  $.058$ , respectively). In addition, there was a significant increase over time in TP ( $P = .000$ ).

### 3.3. Group differences in the relations between RTL and blood chemistries

To examine relations between telomere length and blood chemistry, general linear model analysis was performed for RTL based on blood chemistry, group, and time (Table 3). There were significant interactions between group and several blood chemistry parameters: TG, HDL, GOT, and GPT ( $P = .007$ ,  $.041$ ,  $.020$ , and  $.019$ , respectively, Table 3). As plasma TG, GOT, or GPT increases, RTL decreases in the control group, while the relations seemed reversed in the EXP group (Fig. 2A, C, D). As plasma HDL increases, RTL decrease in the EXP group, while it seemed reversed in the control group (Fig. 2B). Pearson correlation analysis for each group showed this trend more clearly. In the control group, RTL was significantly negative-correlated with TG [ $r(29) = -.52$ ,  $P = .003$ ], GOT [ $r(29) = -.51$ ,  $P = .003$ ], or GPT [ $r(29) = -.47$ ,  $P = .008$ ], while it did not show such associations in the EXP group: [ $r(39) = .25$ ,  $P = .108$ ] for TG; [ $r(39) = .20$ ,  $P = .218$ ] for GOT; [ $r(39) = .23$ ,  $P = .148$ ] for GPT (Fig. 2A, C, D). Correlation of RTL and HDL was negative

**Table 2**

Measurements, repeated measures ANOVA results, and post hoc comparisons of blood chemistries.

	EXP		CTL		rmANOVA			Post hoc comparison			
Blood Chemistries (N <sub>EXP</sub> /N <sub>CTL</sub> )	Pre	Post	Pre	Post	Group	Time	Group*Time	Pre (EXP vs CTL)	Post (EXP vs CTL)	EXP (pre vs post)	CTL (pre vs post)
GLU (20/15)	93.24 ± 3.13	95.09 ± 3.96	102.14 ± 3.61	107.88 ± 4.57	.034*	.119	.417	.072	.042*	.556	.119
TC (20/15)	193.65 ± 8.79	191.60 ± 8.36	193.40 ± 10.15	199.67 ± 9.65	.758	.572	.269	.985	.532	.674	.270
LDL (20/15)	118.40 ± 7.99	116.95 ± 7.99	115.60 ± 9.23	127.27 ± 9.22	.751	.140	.060	.820	.404	.745	.029*
TG (20/15)	103.23 ± 19.83	105.08 ± 20.95	143.43 ± 22.90	152.26 ± 24.19	.151	.574	.713	.194	.150	.882	.539
HDL (20/15)	58.48 ± 2.56	58.41 ± 2.61	51.82 ± 2.96	53.75 ± 3.02	.125	.572	.546	.099	.251	.977	.440
TP (20/15)	7.28 ± 0.08	7.54 ± 0.06	7.53 ± 0.10	7.79 ± 0.07	.015*	.000***	.988	.058	.014*	.002**	.006**
GOT (20/15)	22.20 ± 1.93	21.35 ± 2.04	24.67 ± 2.23	25.73 ± 2.36	.206	.942	.518	.408	.170	.661	.633
GPT (20/15)	17.00 ± 3.48	19.85 ± 3.97	21.53 ± 4.02	25.67 ± 4.58	.346	.059	.721	.400	.344	.231	.135
LDH (20/9)	181.45 ± 8.53	186.65 ± 17.82	179.89 ± 12.72	184.33 ± 26.56	.908	.800	.984	.920	.943	.806	.888
BUN (20/15)	12.96 ± 0.60	13.07 ± 0.88	12.26 ± 0.69	13.59 ± 1.01	.928	.228	.307	.448	.699	.885	.143
CREA (20/15)	0.80 ± 0.04	0.81 ± 0.03	0.86 ± 0.05	0.87 ± 0.04	.242	.581	.937	.324	.227	.717	.676

The EXP or CTL column values indicate mean ± standard error of blood chemistries for each group and time. The repeated measures analysis of variance (rmANOVA) or Post Hoc Comparison column values indicate the  $P$  values.

BUN = blood urea nitrogen, CTL = control, CREA = creatinine, EXP = experiment, GLU = glucose, GOT = glutamic oxaloacetic transaminase, GPT = glutamate pyruvate transaminase, HDL = high-density lipoprotein cholesterol, LDH = lactate dehydrogenase, LDL = low-density lipoprotein cholesterol, Post = post-measurement, Pre = pre-measurement, TG = triglyceride, TP = total protein, TC = total cholesterol.

\* $P \leq .05$ ,

\*\* $P \leq .01$ ,

\*\*\* $P \leq .001$ .

**Table 3****General linear models of blood chemistries for relative telomere length.**

Blood chemistries EXPpre, EXPpost/ CTLpre, CTLpost	GLU 20, 21/15, 16	TC 20, 21/15, 16	LDL 20, 21/15, 16	TG 20, 21/15, 16	HDL 20, 21/15, 16	TP 20, 21/15, 16	GOT 20, 21/15, 16	GPT 20, 21/15, 16	LDH 20, 21/9, 16	BUN 20, 21/15, 16	CREA 20, 21/15, 16
	P value										
Model Summary	.651	.885	.876	.222	.413	.755	.272	.265	.802	.931	.931
Intercept	.000***	.000***	.000***	.000***	.000***	.000***	.000***	.000***	.000***	.000***	.000***
Blood Chemistry	.125	.488	.413	.676	.291	.187	.948	.470	.615	.869	.678
age	.151	.144	.141	.170	.096	.123	.090	.053	.197	.181	.196
Group	.103	.666	.763	.041*	.034*	.757	.038*	.076	.832	.949	.663
(ref. = CTL)											
Time (ref. = pre)	.649	.877	.799	.640	.771	.661	.921	.711	.808	.575	.898
Group * Blood	.105	.609	.680	.007**	.041*	.730	.020*	.019*	.902	.978	.597
Chemistry											
Time * Blood	.633	.835	.730	.687	.714	.635	.923	.439	.853	.612	.944
Chemistry											
Group * Time	.742	.675	.830	.815	.990	.974	.528	.280	.469	.899	.871
Group * Time *	.797	.573	.661	.793	.836	.961	.348	.298	.383	.779	.970
Blood Chemistry											

P values of general linear models for predicting telomere length, including experimental design variables and covariate of age. Each model includes main effects and interactions with blood chemistries as indicated by the column headings.

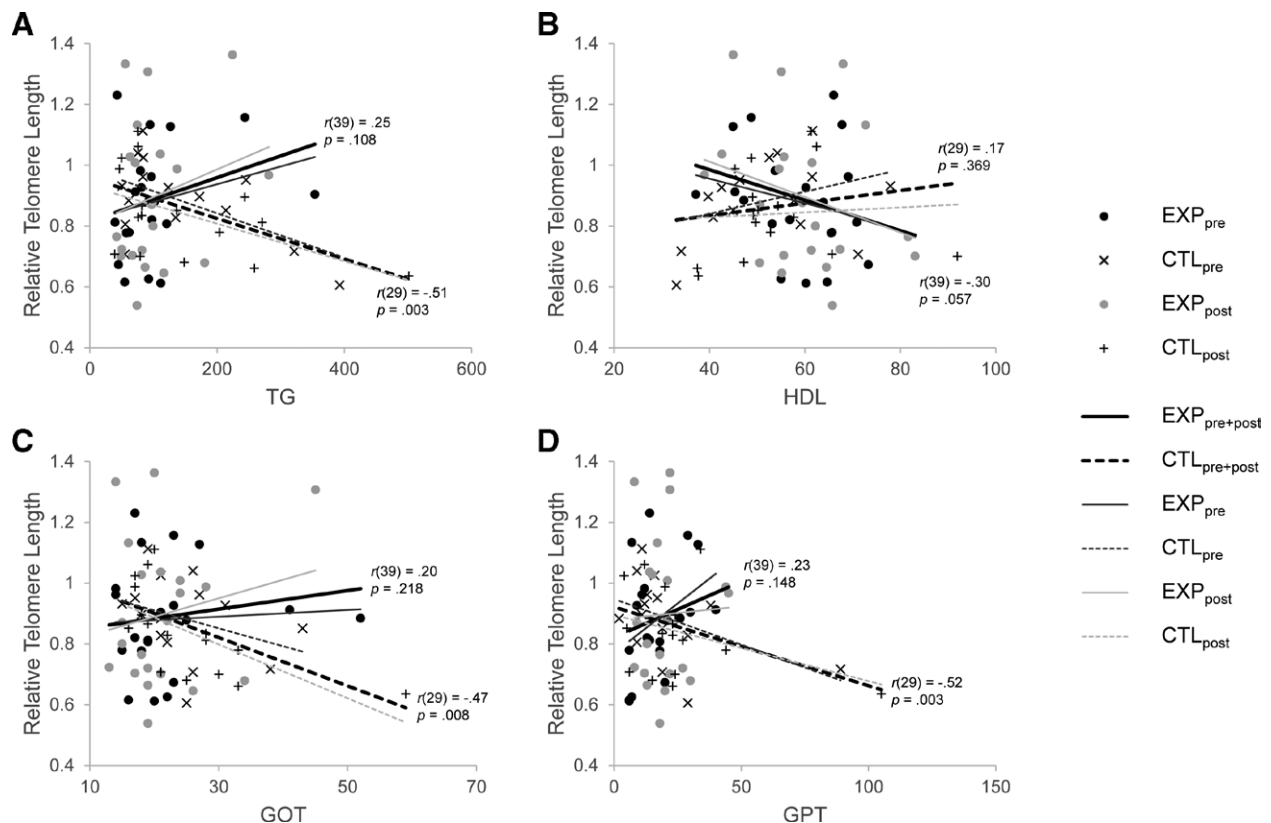
BUN = blood urea nitrogen, CREA = creatinine, CTL = control, EXP = experiment, GLU = glucose, GOT = glutamic oxaloacetic transaminase, GPT = glutamate pyruvate transaminase, HDL = high-density lipoprotein cholesterol, LDH = lactate dehydrogenase, LDL = low-density lipoprotein cholesterol, TC = total cholesterol, TG = triglyceride, TP = total protein.

Ref. indicates the reference category for categorical predictors.

\* $P \leq .05$ ,

\*\* $P \leq .01$ ,

\*\*\* $P \leq .001$ .



**Figure 2.** Group differences in relationships of relative telomere length with TG, HDL, GOT and GPT. Trend lines between telomere and TG, HDL, GOT and GPT in the EXP group (solid line) and the control group (dotted line). Pearson correlation values between relative telomere length and blood chemistries by the group are indicated next to solid lines. CTL = control, EXP = experiment, GOT = glutamic oxaloacetic transaminase, GPT = glutamate pyruvate transaminase, HDL = high-density lipoprotein cholesterol, Pre = pre-measurement, Post = post-measurement, RTL = relative telomere length, TG = triglyceride.

in the EXP group with a tendency of significance [ $r(39) = -.30$ ,  $P = .057$ ], while it was positive without significance in the control group [ $r(29) = .17$ ,  $P = .369$ ] (Fig. 2B). There was no significant result of general linear model analysis for RTL with other blood chemistries (Table 3).

### 3.4. Self-reported questionnaires

Two-way mixed-design ANOVA was performed for the questionnaires (Table 4). Depression ( $P = .022$ ) and stress ( $P = .027$ ) levels were lower while EI ( $P = .031$ ) levels were higher in the EXP group compared to the control group. Pittsburgh Sleep Quality Index tended to be slightly lower ( $P = .089$ ) in the EXP group (Table 4). There was no significant change in group by time interaction effect in all the indicated questionnaires. Patient Health Questionnaire-15 exhibited a marginal trend toward significance in group by time interaction ( $P = .096$ ). SR exhibited a tendency of significance in time effect ( $P = .090$ ). General linear model analysis was performed to examine associations between RTL and questionnaires, however there were no significant correlations observed (Table S3, <http://links.lww.com/MD/H454>).

## 4. Discussion

### 4.1. Different telomere length dynamics between the groups

In this study, the average age of participants was 50-years old: 48.33 (meditation) and 51.18 (control) years old, without group difference (Table 1). A previous study has suggested that meditation is effective in slowing the progress of biological aging in midlife.<sup>[6]</sup> In a general population of non-meditators, epigenetic measurements of biological age showed a distinct difference between people over the age of 52 compared with those below 52. In contrast, biological age was maintained in meditation experienced (of  $\geq 3$  years) people of same age range. Similarly, in other studies whose participants' ages were around 50 (Mean age of participants = 49,<sup>[45]</sup> 50,<sup>[7]</sup> 55<sup>[8]</sup>), TL was maintained or increased within of the study period (approximately 1–3 months) in the meditation group, while it was reduced in the control group. The mean age of the participants in our study was 50 and we observed similar results (Tables 2 and 3, Fig. 1). In the meditation-naïve control group, RTL was found to be significantly reduced within the study period (3 months), while it was maintained in the meditator group. In the above studies whose participants' mean age was around 50, meditation practice was observed to be correlated with the increase or maintenance of TL. However, in various studies with participants of a younger mean age, meditation did not appear to affect TL. In 1 study where the mean age of participants

was 40.5 years old, meditation naïve adults were enrolled in a 9-month training program consisting of 3 distinct mental training modules of 3 months each, but no effects of the training on RTL were observed.<sup>[10]</sup> In another study where the participants' mean age was 27, no significant changes in TL were observed between groups subjected to MBSR or music therapy-based stress reduction program for 8 weeks.<sup>[9]</sup> After midlife, aging markedly accelerates compared to younger adults. Meditation may increase the threshold for stress response in the brain, altering the consecutive responses mediated by multiple systems including the autonomic nervous system, HPA axis, and gut-brain axis, as well as the endocrine and immune systems. These changes perpetuate a virtuous circle for bodily health,<sup>[1]</sup> potentially contributing to the delay of aging progression.

In this study, the RTL was significantly reduced in the control group after the 3-month daily life routine (Fig. 1, Table 3). Previous studies also exhibited significantly reduced telomere length in meditation naïve midlife adults that led a 12-week daily life routine.<sup>[45]</sup> Telomere length in breast cancer survivors was maintained in mindfulness-based cancer recovery or supportive-expressive group therapy, while it was shortened in the control group of usual care for 8 weeks.<sup>[8]</sup> Epigenetic marks change with aging,<sup>[46]</sup> with a transient increase in histone acetylation during midlife being previously noted.<sup>[46]</sup> A day of intensive practice of mindfulness meditation in meditation-experienced subjects showed a significant decrease in the expression of histone deacetylase genes (HDAC 2, 3, 9), and a significant change in the global modification of histones (H4ac; H3K4me3), compared to the meditation-naïve control.<sup>[47]</sup> Considering the dynamic variation of epigenetic modalities affecting biological age in blood cells in such a short period of 1 day, and the change in telomere length within 12 or 8 weeks of daily life in previous studies, the 3-month study period here is sufficiently long to observe significant telomere length changes.

In this study, the participants in the experimental group had a meditation experience with a mean of  $10.33 \pm 1.11$  years,  $42.14 \pm 6.43$  minutes, and  $2.52 \pm 0.44$  times daily practice, however it was self-reported and not checked or controlled. During the 3-months study period, the practices were monitored and conducted by professional trainers. Therefore, there was a question of whether properly conducted intensive training would have an effect on the measurements studied here. There were no significant differences in RTL between the 2 groups at the beginning of the study. However, RTL was reduced in the control group while it was maintained in the experimental group after the 3-month period (Fig. 1). In a previous study, participants involved in a 1-month retreat with a high intensity of Insight meditation training (10 hours a day of meditation), had an increased telomere length compared to meditation-experienced people who did not participate in the intensive retreat,<sup>[7]</sup> suggesting that training intensity affects telomere length.

**Table 4**  
Measurement and repeated measures ANOVA results of self-reported questionnaires.

Blood chemistries (NEXP/NCTL)	EXP		CTL		rmANOVA		
	Pre	Post	Pre	Post	Group	Time	Group*Time
PSQI (16/13)	6.00 $\pm$ 0.80	5.38 $\pm$ 0.80	7.54 $\pm$ 0.88	7.85 $\pm$ 0.89	.089	.669	.215
PHQ-15 (17/14)	6.29 $\pm$ 1.21	5.24 $\pm$ 1.17	6.57 $\pm$ 1.34	7.21 $\pm$ 1.29	.514	.677	.096
Dep (17/14)	2.24 $\pm$ 0.63	1.53 $\pm$ 0.69	4.29 $\pm$ 0.69	3.64 $\pm$ 0.76	.022*	.154	.946
Anx (17/14)	1.47 $\pm$ 0.60	1.41 $\pm$ 0.75	2.93 $\pm$ 0.66	3.29 $\pm$ 0.83	.076	.743	.647
Str (17/14)	2.76 $\pm$ 0.73	2.24 $\pm$ 0.77	5.14 $\pm$ 0.80	4.50 $\pm$ 0.85	.027*	.260	.912
EI (17/13)	79.12 $\pm$ 3.05	82.53 $\pm$ 2.78	71.31 $\pm$ 3.48	71.54 $\pm$ 3.18	.031*	.258	.322
SR (17/14)	47.06 $\pm$ 2.29	52.06 $\pm$ 2.05	44.79 $\pm$ 2.52	45.14 $\pm$ 2.26	.118	.090	.139

The EXP or CTL column values indicate mean  $\pm$  standard error of blood chemistries for each group and time. Values in the rmANOVA column indicate  $P$  values.

Anx = anxiety, CTL = control, Dep = depression, EI = emotional intelligence, EXP = experiment, PHQ-15 = Patient Health Questionnaire-15, Post = post-measurement, Pre = pre-measurement, PSQI = Pittsburgh Sleep Quality Index, SR = self-regulation, Str = stress.

\* $P \leq .05$ .

Similarly, previous also show that the type of meditation may also affect changes in RTL. A 6-week Loving-Kindness Meditation group, whose main features are related with warmth, kindness as well as social connection, maintained the telomere length, while the mindfulness meditation, whose main focus is related with viewing one's experience with more clarity showed significantly reduced telomere length similar to the control group after the 6-week,<sup>[45]</sup> suggesting that the kind of practice might make a difference in the effects.

#### 4.2. Improved psychological features in the MBI group

The MBI which used in this study is the combination of moving meditation (i.e., *Brain Wave Vibration* meditation) and focused attention meditation (i.e., *Sangdahnjeon* meditation). According to previous investigations, *Brain Wave Vibration* meditation significantly reduces depression, anxiety, stress,<sup>[26,48–50]</sup> improves EL,<sup>[48,51]</sup> and global sleep quality.<sup>[24]</sup> In the current study, the self-report questionnaires exhibited consistent results with the previous reports of the *brain wave vibration* meditation: depression, anxiety, stress, EL, and sleep quality exhibited improvements with a significant difference or a tendency of significance in the experimental group compared to the control group (Table 4).

#### 4.3. Distinct correlations of RTL with TG/GPT/GOT/HDL between the groups

In the blood chemistry analysis, GLU level was higher in the control group than the experimental group (Table 2). In the questionnaires, the experimental group reported a significant reduction in stress and depression compared to the control group (Table 4), implying a potentially lower HPA axis activity, contributing to a lower cortisol secretion<sup>[52]</sup> in the experimental group, compared to the control group. In a cohort study for 3270 individuals, raised evening cortisol or flattened diurnal cortisol slope was prognostic of the onset of type 2 diabetes or impaired fasting glucose after about 10 years,<sup>[53]</sup> suggesting the effects of stress response on glucose metabolism. Therefore, the lower GLU level in the experimental group may be indicative of changes in GLU metabolism resulting from lower stress levels.

Interestingly, we found LDL level behaves differently between groups by time: a significant increase in the control group at the post-intervention compared to the pre-intervention, while no changes by time were observed in the meditation group (Table 2). This result is similar with those observed in previous studies. In a 16-week RCT for healthy obese postmenopausal women (mean age 54), the intervention (yoga) group significantly reduced LDL, while the control group significantly increased it.<sup>[30]</sup> In an 8-week RCT for patients suffering from hypertension/diabetes (mean age 69), the meditation intervention group exhibited significantly reduced LDL, while the control group showed an increase.<sup>[22]</sup> In another 6-month RCT for patients with coronary artery disease, yoga practitioners also exhibited a higher reduction rate in LDL than the control group.<sup>[54]</sup> These results suggest that the blood LDL level might be one of the physiological factors that are affected by MBI.

The relations of RTL with blood components (i.e., TG, GPT, GOT, HDL) were significantly different in the experimental group compared to the control group (Table 3 and Fig. 2). Telomere length was previously found to be inversely correlated with TG in type 2 diabetes males<sup>[20]</sup> as well as healthy adults.<sup>[21]</sup> In another study, people with elevated GPT (in non-alcoholic fatty liver disease) had a significantly lower TL than people with normal GPT (nonalcoholic fatty liver disease subjects),<sup>[55]</sup> suggesting an inverse correlation between TL and GPT. Consistently with this, in the control group, there was a significant inverse correlation between RTL and TG/GPT (Fig. 2A, D, dotted lines). In contrast, these inverse correlations were not

observed in the experimental group (Fig. 2A, D, lines). Similarly, in the correlation between RTL and GOT, we also found a significant inverse correlation in the control group (Fig. 2C, dotted lines), but no correlation in the experimental group (Fig. 2C lines). In the correlation between RTL and HDL, there was a tendency of significance in the inverse correlation in the control group, however no correlation was found in the experimental group (Fig. 2B). These results indicate that the MBI may change the correlation of the aging extent of peripheral blood cells with blood chemistry.

The current study design is not a randomized controlled study but a quasi-experimental study. Therefore, in order to understand the direct cause and effects of the intervention, a randomized controlled trial is expected to be performed with a larger number of participants. Although significantly more work has to be done to understand the underlying mechanism of the MBI, the current results support the efficacy of MBI training in modifying aging process in molecular cellular level together with beneficial changes in psychological dimensions in midlife populations.

#### Author contributions

All authors have read and agreed to the published version of the manuscript.

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