

# Effects of Meditation and Music-Listening on Blood Biomarkers of Cellular Aging and Alzheimer's Disease in Adults with Subjective Cognitive Decline: An Exploratory Randomized Clinical Trial

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## Abstract.

**Background:** Telomere length (TL), telomerase activity (TA), and plasma amyloid- $\beta$  (A $\beta$ ) levels have emerged as possible predictors of cognitive decline and dementia.

**Objective:** To assess the: 1) effects of two 12-week relaxation programs on TL, TA, and A $\beta$  levels in adults with subjective cognitive decline; and 2) relationship of biomarker changes to those in cognitive function, psychosocial status, and quality of life (QOL).

**Methods:** Participants were randomized to a 12-week Kirtan Kriya meditation (KK) or music listening (ML) program and asked to practice 12 minutes/day. Plasma A $\beta$ (38/40/42) and peripheral blood mononuclear cell TL and TA were measured at baseline and 3 months. Cognition, stress, sleep, mood, and QOL were assessed at baseline, 3 months, and 6 months.

**Results:** Baseline blood samples were available for 53 participants (25 KK, 28 ML). The KK group showed significantly greater increases in A $\beta$ <sub>40</sub> than the ML group. TA rose in both groups, although increases were significant only among those with higher practice adherence and lower baseline TA. Changes in both TL and TA varied by their baseline values, with greater increases among participants with values  $\leq$ 50th percentile ( $p$ -interaction  $<0.006$ ). Both groups improved in cognitive and psychosocial status ( $p \leq 0.05$ ), with improvements in stress, mood, and QOL greater in the KK group. Rising A $\beta$  levels were correlated with gains in cognitive function, mood, sleep, and QOL at both 3 and 6 months, associations that were particularly pronounced in the KK group. Increases in TL and TA were also correlated with improvements in certain cognitive and psychosocial measures.

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**Conclusion:** Practice of simple mind-body therapies may alter plasma A $\beta$  levels, TL, and TA. Biomarker increases were associated with improvements in cognitive function, sleep, mood, and QOL, suggesting potential functional relationships.

**Keywords:** Alzheimer's disease, cognition, memory complaints, mind-body therapy, mood, plasma amyloid- $\beta$ , quality of life, sleep, subjective cognitive impairment, telomerase, telomeres

## INTRODUCTION

Several blood biomarkers implicated in the development of cognitive impairment and dementia have emerged as potentially useful predictors of cognitive decline and dementia, and as potential targets for therapeutic intervention. For example, telomeres, which cap the ends of linear chromosomes and act as a 'mitotic clock' [1, 2], may have promise as biomarkers of cognitive aging [1–3]. Shortened leukocyte telomere length (TL) has been associated with accelerated aging [3], as well as with vascular, metabolic, and psychological risk factors for AD [4–7]. Reduced TL has also been linked to increased risk for cognitive decline, mild cognitive impairment (MCI) [8, 9], and Alzheimer's disease (AD) [10] in some longitudinal studies, although others have reported no association [5, 11, 12]. There is also accumulating evidence suggesting a link between human telomerase activity (TA) and pathological mechanisms underlying AD [13]. Other blood biomarkers of emerging interest include plasma amyloid- $\beta$  (A $\beta$ ) levels, which have been associated with risk for cognitive decline and conversion to dementia. However, studies to date have produced inconsistent results. While some longitudinal studies have shown significant inverse associations between blood A $\beta$  levels and subsequent cognitive decline or progression to dementia [14–17], others have indicated positive [18] or no associations [19, 20].

Recent epidemiologic studies suggest TL, TA, and plasma A $\beta$  levels vary with age and other demographic characteristics [21], and may be modified by lifestyle and health-related factors linked to dementia risk. The latter include insulin resistance and diabetes [22, 23], impaired sleep [24–26], and psychological distress [27–29], as well as physical activity and other behavioral factors [29–31]. Findings from recent clinical trials suggest that lifestyle interventions and certain mind-body therapies, including meditation [29, 32] may favorably influence both TL and TA [29, 32, 33]. While there are limited data suggesting that lifestyle interventions may alter plasma A $\beta$  levels [34, 35] and other potential AD biomarkers in the blood [36], research to date remains sparse, and

the effects of mind-body therapies on plasma A $\beta$  or other indices of A $\beta$  burden in those with preclinical memory loss remain unknown. In this exploratory randomized clinical trial (RCT), we assessed the effects of a 12-week Kirtan Kriya meditation (KK) versus a 12-week music listening (ML) program on change in markers of cellular aging (TL, TA) and plasma A $\beta$  levels in older adults with subjective cognitive decline (SCD). In addition, we examined the relationship of changes over time in these biomarkers to improvements in measures of cognitive function, psychological status, sleep, and quality of life (QOL).

## MATERIALS AND METHODS

Participant eligibility criteria, trial design, study procedures, and interventions have been described in detail elsewhere [37] and are outlined briefly below. The study was approved by the West Virginia University Institutional Review Board.

### *Study participants*

Study participants were 60 independently living older adults experiencing SCD, recruited using brochures and flyers placed in health care, community, and workplace settings, as well as institutional intranet and email advertising. Inclusion criteria were as follows: English-speaking adults at least 50 years of age with either 1) SCD defined as meeting six criteria consistent with expert reviews and prospective studies available at the time [38–41]; or 2) physician confirmed diagnosis of MCI. SCD criteria included: 1) presence of subjective cognitive deficits within the past 6 months; 2) able to give an example in which memory/cognitive problems occur in everyday life; 3) frequency of memory problems at least once/week; 4) absence of overt cognitive deficits (e.g., inability to follow simple directions or to complete questionnaires) or previous diagnosis of cognitive impairment or dementia; 5) belief that one's cognitive capacities have declined compared to 5 or 10 years ago; and 6) expressed worry regarding one's memory problems, a factor shown to further increase risk of progression to MCI and AD [41, 42]. Excluded

from the study were those who: 1) recently (within the last 6 weeks) changed dosage of cholinesterase inhibitors (e.g., donepezil (Aricept)) or psychotropic medication (e.g., tricyclics); 2) practiced meditation or another relaxation technique within the past year; 3) had received chemotherapy treatment within the past 10 years; 4) had a history of psychotic or schizophrenic episodes, major neurologic diagnosis (e.g., Parkinson's, stroke) or other condition that might impair cognition or confound assessments; or 5) recently (within the last 3 months) experienced serious physical trauma or received a diagnosis of a serious chronic health condition requiring medical treatment and monitoring. All eligibility criteria were evaluated during the telephone and baseline screening process. Study buddies willing to attend all assessment visits were required for participants with a diagnosis of MCI (of whom none were recruited or enrolled [37]) and encouraged for those who were concerned about their ability to fully understand the consent process or complete the questionnaires.

#### *Assessments*

Following provision of written informed consent, baseline assessments were conducted. We gathered information on demographic factors, lifestyle, and anthropometric characteristics, current medication and supplement use, and medical history. In addition, blood samples were collected, processed, and stored as described below. Cognitive function, psychosocial status, and quality of life were also evaluated, as were treatment expectancy and other factors (see below).

#### *Blood collection and processing and biomarker assay*

To permit assessment of possible changes in A $\beta$  levels and markers of cellular aging, blood draws were performed at baseline and 3 months. To minimize discomfort, blood samples were collected by phlebotomists experienced with geriatric, pediatric, and/or cancer patients. In addition, we followed a two-stick maximum policy, which aided in further reducing participant discomfort and anxiety [37]. Using a winged (butterfly) blood collection set, 10 ml of whole blood was collected into a 6 ml EDTA and a 4 ml sodium citrate tube. All blood samples were processed within 30 min of collection and immediately frozen at  $-80^{\circ}\text{C}$  until assay. Samples were thawed only immediately prior to analysis.

*Plasma collection, handling, and storage.* To allow measurement of plasma A $\beta$  peptides, blood was

drawn into EDTA tubes and immediately centrifuged ( $378 \times g$  (1400 rpm using Beckman Coulter Allegra X-22R centrifuge) for 10 min at  $4^{\circ}\text{C}$ ); plasma was separated from contact with cells immediately after centrifugation, aliquoted into 100  $\mu\text{L}$  prelabeled 0.5 mL plastic microcentrifuge tubes, and stored at  $-80^{\circ}\text{C}$  until assay. Assays were batched, and all samples run at the same time to minimize inter-assay variability. All analyses were conducted by the WVU Flow Cytometry & Single Cell Core Facility.

For collection of peripheral blood mononuclear cells to assess TL and TA, 4 ml of whole blood were drawn into a 4 mL Cell Preparation Tube sodium citrate tube and centrifuged at  $378 \times g$  (1324 rpm using Sorvall Legend RT)  $\times$  15 min at room temperature. Following centrifugation, the cell layer was immediately collected via pipette and transferred to a 15 mL conical centrifuge tube, resuspended with sufficient 1X phosphate-buffered saline to a volume of 15 mL and centrifuged at  $1800 \times g$  (2888 rpm using Sorvall Legend RT)  $\times$  20 min at room temperature. Following removal of the supernatant, each sample was labeled and stored at  $-80^{\circ}\text{C}$  until assay. Following completion of data collection, extract samples were sent by FedEx overnight on dry ice to Capital Biosciences, Inc. (Gaithersburg, MD) for analysis.

#### *Plasma A $\beta$ quantification (WVU Flow Cytometry & Single Cell Core Facility)*

Plasma levels of A $\beta$  peptides (A $\beta_{38}$ , A $\beta_{40}$ , and A $\beta_{42}$ ) were analyzed using Meso Scale Diagnostics (MSD) Multi-spot A $\beta$  validated Triplex Assay (V-PLEX A $\beta$  Peptide Panel 1 (6E10) Kit, Meso Scale Diagnostics, LLC, Rockville MD) according to the kit manufacturer's instructions. Briefly, plasma samples were thawed at room temperature and immediately placed on ice. Diluent 35 (MSD, LLC) was added to each well of the MSD plate and the plate was incubated for 1 h at room temperature with shaking. After washing the plate with phosphate buffered saline (PBS) containing 0.05% Tween 20, diluted anti-A $\beta$ 6E10 antibody was added to each well. Samples were diluted 1:2 with diluent 35 and added to the appropriate wells. The plate was then incubated for 2 h at room temperature with shaking. Finally, the plates were washed with wash buffer and 2x Read buffer T was added to each well. Plates were read on an MSD Sector Imager 2400 within 5 min of the 2x Read buffer T addition. All participant samples were run in duplicate on the same plate. Internal standards were used to control for plate-to-plate variation. To generate standard curves, each plate had a set of

calibrators run in duplicate. A $\beta_{38}$  detectable range was from 2.63 pg/ml to 10,800 pg/ml. A $\beta_{40}$  detectable range was from 3.64 pg/ml to 14,900 pg/ml. A $\beta_{42}$  range was from 0.337 pg/ml to 1,380 pg/ml.

*Measurement of telomere length and telomerase activity (Capital Biosciences, Inc., Gaithersburg, MD)*

Telomerase activity was quantified using the telomeric repeat amplification protocol (TRAP) with a well validated commercial kit (TRAPeze<sup>®</sup> Kit RT, Millipore, Catalog No. S7710) and according to the manufacturer's protocol. The TRAPeze<sup>®</sup> RT Telomerase Detection Kit is a one buffer, two enzyme system utilizing polymerase chain reaction (PCR) and Amplifluor<sup>®</sup> primers. In the first step of the reaction, the telomerase enzyme adds a number of telomeric repeats (GGTTAG) onto the 3' end of a substrate oligonucleotide (TS). In the next steps, the extended products are amplified by the second enzyme, Taq Polymerase, using PCR with the TS and fluorescein-labeled Amplifluor<sup>®</sup> RP (reverse) primers. This generates a fluorescent ladder of products with 6 base increments starting at 61 nucleotides: 61, 67, 73, 79, etc. The fluorescence emission produced is directly proportional to the amount of TRAP products generated.

In addition to the 5X TRAPeze<sup>®</sup> RT Reaction Mix, a second 5X TRAPeze<sup>®</sup> RT Control Reaction Mix including a TSK control template provided an artificial PCR control to assess PCR inhibitor status and a positive control for PCR amplification. Standard curves using the accompanying TSR8 quantitation control allowed quantitation of TA relative to TSR8 product amplification.

Briefly, cell pellets of the samples were resuspended in CHAPS lysis buffer and protein concentrations were determined using Pierce BCA Protein Assay Kit (ThermoFisher, Cat. 23225). For the TRAP assay, all samples were diluted to a final concentration of 750 ng/ $\mu$ l.

Heat-inactivated extracts controls for each sample were prepared by incubation of aliquots at 85°C for 10 min. The TRAP assay was performed using an ABI 7500 Fast Real-Time PCR system. TA, expressed in TSR8 copies for each sample, was calculated by interpolation of Ct values obtained for each replicate using a standard curve. Replicates with Ct values higher than the "no telomerase control" were considered negative and equal to zero TSR8 copies. Interpolation

from the standard curves was performed using GraphPad Prism with setting for 95% confidence intervals and automatic removal of outliers.

*Telomere length assay.* Total genomic DNA was extracted from cell lysates using the following protocol. Aliquots of CHAPS lysate were diluted with DNA lysis buffer containing 1 mg/ml Proteinase K (Qiagen) and incubated at 56°C for 20 min. DNA was precipitated by isopropanol and pelleted by centrifugation at 14000 rpm for 5 min. DNA pellets were washed twice with 70% ethanol, dried, and dissolved in TE buffer. DNA concentration was determined by UV spectrophotometer at 260 nm. TL was estimated by the qPCR method described by O'Callaghan and Fenech (Biological Procedures Online 2011, 13:3; <http://www.biologicalproceduresonline.com/content/13/1/3>). Synthetic oligomers for telomere sequence and single copy gene 36B4 were used as standard templates. Standard curves were generated by performing serial template dilutions of ( $10^{-1}$  to  $10^{-6}$ ). Plasmid DNA (PUC19) was added to each standard to maintain 20 ng of total DNA per reaction. qPCR with TEL or 36B4 primers were done for each sample using Power SYBR<sup>®</sup> Green PCR Master Mix (ThermoFisher, Cat. 4367659). Total telomere sequences and copies of 36B4 per diploid genome were calculated by interpolation from the corresponding standard curves.

Interpolation from the standard curves was performed using GraphPad Prism with setting for 95% confidence interval and automatic removal of outliers. Absolute telomere length (aTL) was calculated by dividing total telomere sequence in kb by diploid genome copy number.

*Measures of cognitive function, psychosocial status and quality of life (QOL)*

We assessed change in memory and cognitive function, psychosocial status, sleep, and QOL using established, well-validated instruments. Cognitive measures included those to evaluate memory function (Memory Functioning Questionnaire (MFQ) [43]), executive function (Trail Making Test Parts A and B (TMT) [44]), and psychomotor speed, attention, and working memory (the 90-s Wechsler Digit-Symbol Substitution Test (DSST) [45]). Psychosocial measures included self-report instruments to assess mood (65-item Profile of Mood States [46]), well-being (Psychological Well-Being Scale [47]), perceived stress (10-item Perceived Stress Scale [48, 49]), sleep quality (Pittsburgh Sleep Quality Index [50]), and

health-related QOL (36-item MOS Short Form-36 [47]). Assessments were performed at baseline, 3 months, and 6 months (3 months post-intervention).

#### *Other measures*

To determine *expectation of benefit*, participants completed the 6-item Credibility/Expectancy Questionnaire [51] following their first intervention practice session. Participants completed daily home practice logs, collected at the follow-up assessment visits to allow tracking of adherence. Changes in physical activity, medication, and/or supplement use were assessed at each follow-up visit using a form specifically designed for this purpose. In addition, participants were also asked to complete a 3- and 6-month study evaluation questionnaire adapted from that used in our previous studies [52, 53], which included a single 5-point Likert scale item to assess change in concerns regarding memory. All assessors were blinded to participant treatment assignment.

#### *Randomization and treatment allocation*

Eligible participants were randomly assigned to one of the two groups described below, in a 1:1 ratio using a randomly varying block randomization method to ensure equal distribution between groups [54]. Following baseline assessment, the next statistician-prepared, sequentially numbered, sealed opaque envelope containing the treatment assignment was given to the participant by the consenting team member, who had no advance knowledge of the group allocation schedule.

#### *Interventions*

Participants were instructed to engage in their assigned practice while seated comfortably with eyes closed, for 12 min daily for 12 weeks (84 practice sessions total) and to record every practice session daily on the home practice log, along with any comments; they were also asked to record any practice sessions they completed during the 3-month, practice-optional, post-intervention period. Following randomization, participants were provided 30–45 min of one-on-one, onsite training and practice in their assigned program (see below), facilitated by a trainer familiar with both study programs and experienced in teaching a range of mind-body skills. In addition, each participant received a short reference guide, a program CD, and a portable CD player for home use. Within the first week of the

intervention, the trainer called each participant to provide additional instruction and clarification as needed; she remained available thereafter to address any additional issues that arose over the course of the intervention.

#### *Kirtan Kriya (KK) meditation program*

A beginner meditation technique, KK is easy to learn and practice, yet engages several areas of the brain. The meditation includes repeated chanting of the ‘Sa-Ta-Na-Ma’ mantra, while touching the thumb to each fingertip in sequence with the chant, and visualizing energy entering the top of the head and exiting between the eyebrows in an ‘L’. The KK CD contained an introductory track with detailed instructions regarding the KK practice and five 12-min meditation tracks, including three guided sessions.

#### *Music listening (ML) program*

Participants were instructed to listen to 12 minutes of relaxing instrumental music from their choice of one of six classical composers each day. They were asked to sample each composer at least once during the 12 weeks. The ML CD contained selections from Bach, Beethoven, Debussy, Mozart, Pachelbel, and Vivaldi.

#### *Statistical analysis*

Data analysis was performed using IBM SPSS, Version 23. Baseline differences between the original study sample and those with blood samples available; between intervention groups; and between dropouts and non-dropouts were assessed using chi-square (for categorical variables), independent samples *t*-tests (for normally distributed continuous variables), or Mann-Whitney U tests (for ordinal or continuous variables with evidence of skewing); a *p* value of  $\geq 0.1$  was used for determining baseline differences. Potential differences between treatment groups were analyzed using chi-square for retention, and one-way ANOVA for treatment expectancies and adherence. In preliminary assessments, within-group changes over time at 3 and 6 months were assessed using ANCOVA with age and baseline scores as covariates; age- and sex-adjusted between-group differences in treatment outcomes were assessed using Repeated Measures ANOVA. Effect sizes were calculated using Cohen’s *d*. As this was a pilot feasibility study, alpha was set at 0.05. Bivariate and age- and sex-adjusted correlations were performed using Pearson’s *r* or Spearman rho. Variables with a non-normal distribution were

log-transformed prior to analysis. Extreme outliers (biomarker values exceeding 4 standard deviations from the mean), were Winsorized and assigned values equal to the next highest/lowest scores.

We performed additional analyses restricted to those most at risk for cognitive decline, including participants: age  $\geq 60$  years with SCD onset within the last 5 years; those with poorer baseline scores on the MFQ ( $< 75$ th percentile) and the TMT-B ( $\geq 88$  s, a cut-off shown to predict subsequent cognitive decline and dementia in a recent study of memory clinic patients with MCI) [55]. We also conducted analyses stratified by high versus low adherence to practice ( $> 33$ rd versus  $\leq 33$ rd percentile), and evaluated the potential modifying influence of sex, age ( $\geq 60$  versus  $< 60$  years), history of depression/anxiety, and baseline biomarker values ( $< 50$ th versus  $\geq 50$ th percentile). To assess the potential influence of change in medication or physical activity, we conducted additional analyses, both adjusting for these factors statistically and excluding, in separate analyses, those reporting changes in medication or exercise.

## RESULTS

Of the sixty adults enrolled in the study (all with SCD), viable baseline blood samples were available for 53 (25 KK and 28 ML). Participant characteristics are given in Table 1. Enrolled participants ranged in age from 50–84 years old (mean (M) = 60.5, SE = 1.2). Participants were predominantly female (87%), non-Hispanic white (94%), and married or cohabiting (64%).

With the exception of prevalence of obesity, which was marginally lower in the KK than in the ML group, the two groups were similar overall in demographics, lifestyle factors, medical history, and in baseline measures of cognitive function, psychosocial status, and QOL (Tables 1 and 2). Changes in medication use and physical activity at the follow-up assessment were also similar between groups ( $ps \geq 0.4$ ). Participants reported experiencing memory problems on average for approximately 3 years (M =  $35.42 \pm 4.2$  months). In 42% of participants, baseline TMT-B scores were 88 or above; mean baseline MFQ scores were  $246.1 \pm 2.9$ , comparable to average scores in adults with amnesic MCI [56], and considerably lower than those reported in community-based studies of older adults [57]. Prevalence of additional modifiable AD risk factors was also high, with 94% of participants reporting at least one, and 66% reporting 2 or more metabolic/vascular risk factors for AD;

overall, prevalence of measured AD risk factors averaged  $2.8 \pm 0.2$  (Table 1).

Baseline TA values were correlated with age ( $r = 0.36$ ,  $p = 0.008$ ), as were baseline measures of memory and cognition ( $rs \geq 0.3$ ,  $ps \leq 0.02$ ). Baseline A $\beta_{40}$  and A $\beta_{42}$  showed inverse associations with BMI ( $rs$  adjusted for age =  $-0.3$ ,  $ps < 0.05$ ). Otherwise, biomarkers were not significantly related to any other demographic, lifestyle, metabolic, psychosocial, or other AD risk factors at baseline. In addition, baseline biomarker values did not differ by history of depression or anxiety, high cholesterol, hypertension, obesity, cancer or other chronic conditions, or by use of medications commonly linked to memory changes.

All participants received the intervention to which they were assigned. Of the 53 participants with baseline blood samples, 48 (91%) completed the 12-week program, and 47 (89%) completed the full 6-month study. Among those completing the 12-week program, viable blood samples for both time points (baseline and 3 months) were available for 45 participants (20 KK, 25 ML). Those with versus without blood samples from both time points were similar in all baseline characteristics ( $ps \geq 0.3$ ).

Program adherence among the analytic sample was high; participants completed an average of 94% (93% KK, 95% ML) of sessions during the 12-week intervention period, and 71% (68% KK, 74% ML) of sessions during the 3-month, practice-optional follow-up period. There were no between-group differences in treatment expectancies (all  $ps \geq 0.2$ ), or in either retention or adherence at any time point ( $ps \geq 0.4$ ). No adverse events were reported by any participant.

*Changes over time in cognitive function, psychosocial status, and QOL* are detailed in Table 3A. At 3 months, both groups showed significant improvements in measures of memory function and cognitive performance, as well as in sleep quality, well-being and multiple domains of mood. The KK group also demonstrated significant reductions in perceived stress and significant improvements in QOL-mental health (QOL-MH) ( $ps \leq 0.05$ ). Gains in all measures were maintained or further increased at 6 months. Improvements in mood, well-being, and QOL-MH were greater in the KK than in the ML group at both 3 and 6 months ( $ps = 0.03$ – $0.09$ ).

Changes in AD biomarkers are detailed in Table 3B. The KK group demonstrated significantly greater increases in plasma A $\beta_{40}$  levels than did the ML group ( $p = 0.04$ ), and tended to show corre-

Table 1  
Participant baseline characteristics: RCT of a 12-week Kirtan Kriya meditation (KK) versus a 12-week music listening (ML) program in older adults with subjective cognitive decline (N = 53)

|  | Overall (N = 53) |                | KK (n = 25) |                | ML (n = 28) |                | p    |
|--|------------------|----------------|-------------|----------------|-------------|----------------|------|
|  | N                | %              | N           | %              | N           | %              |      |
| <b>Demographic characteristics</b>                     |                  |                |             |                |             |                |      |
| Age (range 50–84 y)                                    |                  |                |             |                |             |                |      |
| Mean ± SE  |                  | 60.47 ± 1.17   |             | 60.71 ± 1.38   |             | 60.20 ± 1.63   | 0.81 |
| Female Gender  | 46               | 86.79%         | 23          | 92.00%         | 23          | 82.14%         | 0.29 |
| Race/Ethnicity: Non-Hispanic White                     | 50               | 94.34%         | 23          | 92.00%         | 27          | 96.43%         | 0.49 |
| Education ≥ 12 y                                       | 43               | 81.13%         | 22          | 88.00%         | 21          | 75.00%         | 0.23 |
| Employment status                                      |                  |                |             |                |             |                | 0.47 |
| Employed full or part time                             | 40               | 75.47%         | 21          | 84.00%         | 19          | 76.00%         |      |
| Retired/Homemaker/Other                                | 13               | 24.53%         | 4           | 16.00%         | 9           | 36.00%         |      |
| Marital status   |                  |                |             |                |             |                | 0.37 |
| Married/co-habiting                                    | 34               | 64.15%         | 16          | 64.00%         | 18          | 64.29%         |      |
| Divorced/widowed/separated/single                      | 19               | 35.85%         | 9           | 36.00%         | 10          | 35.71%         |      |
| <b>Lifestyle and health-related factors</b>            |                  |                |             |                |             |                |      |
| Smoking status: Ever smoked                            | 20               | 37.74%         | 11          | 44.00%         | 9           | 32.14%         | 0.37 |
| Caffeinated beverage consumption                       |                  |                |             |                |             |                |      |
| Mean oz consumed/day ± SE                              |                  | 22.83 ± 2.40   |             | 24.31 ± 3.39   |             | 21.52 ± 3.42   | 0.57 |
| Physical activity                                      |                  |                |             |                |             |                | 0.81 |
| None   | 14               | 26.42%         | 7           | 28.00%         | 7           | 25.00%         |      |
| Mean minutes/week ± SE                                 |                  | 117.28 ± 16.25 |             | 112.70 ± 18.47 |             | 121.37 ± 26.29 | 0.79 |
| Body mass index (BMI)                                  |                  |                |             |                |             |                |      |
| Obese (BMI ≥30)  | 24               | 45.28%         | 8           | 32.00%         | 16          | 57.14%         | 0.07 |
| Mean ± SE  |                  | 29.74 ± 1.05   |             | 28.74 ± 1.34   |             | 30.63 ± 1.58   | 0.37 |
| History of diagnosed:                                  |                  |                |             |                |             |                |      |
| Diabetes   | 9                | 16.98%         | 4           | 16.00%         | 5           | 17.86%         | 0.85 |
| Hypertension   | 17               | 32.08%         | 7           | 28.00%         | 10          | 35.71%         | 0.35 |
| High cholesterol                                       | 31               | 58.49%         | 17          | 68.00%         | 14          | 50.00%         | 0.18 |
| Depression   | 18               | 33.96%         | 10          | 40.00%         | 8           | 28.57%         | 0.38 |
| Anxiety  | 16               | 30.19%         | 8           | 32.00%         | 8           | 28.57%         | 0.78 |
| Number of cardiometabolic AD risk factors*             |                  |                |             |                |             |                |      |
| Mean ± SE  |                  | 1.81 ± 0.17    |             | 1.76 ± 0.27    |             | 1.86 ± 0.23    | 0.78 |
| Total number of major modifiable risk factors for AD** |                  |                |             |                |             |                |      |
| Mean ± SE  |                  | 2.77 ± 0.20    |             | 2.72 ± 0.32    |             | 2.82 ± 0.25    | 0.80 |
| Number of medications (regular use) <sup>‡</sup>       |                  |                |             |                |             |                | 0.67 |
| None   | 29               | 54.72%         | 14          | 56.00%         | 15          | 53.57%         |      |
| One  | 13               | 24.53%         | 5           | 20.00%         | 8           | 28.57%         |      |
| Two or more  | 11               | 20.75%         | 6           | 24.00%         | 5           | 17.86%         |      |
| History of hormone replacement therapy <sup>††</sup>   | 17               | 33.33%         | 6           | 23.08%         | 11          | 44.00%         | 0.13 |

\*Including diabetes, hypertension, high cholesterol, obesity (BMI ≥ 30), cardiovascular disease. \*\*Also including history of depression or anxiety disorder, current smoking, and lack of physical activity. †Including those commonly linked to memory changes: Statins, narcotic analgesics, steroids, benzodiazepines, beta blockers, antihistamines, anticonvulsants, tricyclic and other non-SSRI/SNRI antidepressants.

‡‡Percentages calculated in women only.

spondingly lower increases in the  $A\beta_{42}/A\beta_{40}$  ratio ( $p < 0.1$ ). Changes in TL and TA were not significant either within or between groups. However, in the pooled sample, change in TL varied significantly by baseline values, with participants below the 50th centile showing significant increases over time, whereas those with longer TL at baseline showed nonsignificant declines in this biomarker at 3 months ( $3.11 \pm 1.54$  kb versus  $-5.17 \pm 2.96$  kb,  $p$  for interaction = 0.0004). Likewise, rises in TA following the 12-week intervention period were significantly greater in those with lower TA at baseline ( $1745.9 \pm 973.3$  versus  $318.3 \pm 3181.6$  units,  $p$

for interaction = 0.006) (Table 3C). Change in TA also varied significantly by practice adherence, with those reporting weekly average practice adherence over the 33rd percentile ( $> 6.5$  sessions/week) showing significant increases ( $p = 0.04$ ) versus non-significant declines ( $p > 0.2$ ) in those who practiced less ( $3311.7 \pm 342.3$  versus  $-3658.7 \pm 2744.1$ ,  $p$  for interaction = 0.01). Likewise, TA was significantly and positively correlated with practice adherence, even after removal of outliers ( $r_s = 0.5$ ,  $p_s \leq 0.002$ , Fig. 1). Findings were similar when stratified by treatment group. Changes in  $A\beta$  levels were significantly intercorrelated ( $r_s$  adjusted for age and sex = 0.3–0.8).

Table 2

Participant average duration of memory concerns and mean baseline scores on memory and cognitive function tests, and on sleep, stress, mood, well-being, and quality of life questionnaires, stratified by group (Kirtan Kriya meditation (KK) and music listening (ML))

|   | KK (n = 25)<br>Mean (SE) | ML (n = 28)<br>Mean (SE) | p    |
|---|--------------------------|--------------------------|------|
| <b>Biomarkers</b>   |                          |                          |      |
| Telomere length (absolute mean, kb)   | 12.53 (1.28)             | 14.38 (2.61)             | 0.54 |
| Telomerase activity (TSR8 copies)   | 5020.80 (2419.27)        | 1406.69 (856.45)         | 0.11 |
| Plasma A $\beta$ (pg/ml)  |                          |                          |      |
| A $\beta$ 38  | 157.66 (75.9)            | 94.03 (31.39)            | 0.33 |
| A $\beta$ 40  | 231.07 (32.83)           | 215.60 (10.30)           | 0.63 |
| A $\beta$ 42  | 30.39 (9.73)             | 16.76 (2.17)             | 0.19 |
| Ratio A $\beta$ 42/A $\beta$ 40   | 0.10 (0.01)              | 0.08 (0.01)              | 0.14 |
| <b>Cognitive Function</b>   |                          |                          |      |
| <i>Memory Functioning Questionnaire</i>   |                          |                          |      |
| Total   | 238.28 (11.92)           | 248.79 (10.58)           | 0.31 |
| Frequency of Forgetfulness  | 138.32 (5.43)            | 146.86 (5.54)            | 0.32 |
| Seriousness of Forgetting   | 64.00 (4.44)             | 74.75 (4.56)             | 0.12 |
| Retrospective Memory Functioning  | 11.80 (0.72)             | 11.54 (0.63)             | 0.78 |
| Mnemonic Use  | 24.16 (2.13)             | 21.64 (1.82)             | 0.37 |
| <i>Digit Symbol Substitution Test</i>   | 51.32 (1.86)             | 49.50 (1.89)             | 0.50 |
| <i>Trail-making Test (TMT)</i>  |                          |                          |      |
| TMT-A   | 33.83 (1.25)             | 34.61 (2.30)             | 0.78 |
| TMT-B   | 82.92 (7.40)             | 90.57 (7.41)             | 0.41 |
| <i>Months Experiencing Memory Problems</i><br>(range 5 to 180 months, median = 24 months) | 36.52 $\pm$ 7.57         | 32.77 $\pm$ 4.41         | 0.66 |
| <b>Perceived Stress and Sleep Quality</b>   |                          |                          |      |
| <i>Perceived Stress Scale</i>   | 17.80 (1.35)             | 14.93 (1.33)             | 0.14 |
| <i>Pittsburgh Sleep Quality Index, Total</i>  | 9.13 (0.52)              | 8.79 (0.60)              | 0.68 |
| <b>Mood and Well-being</b>  |                          |                          |      |
| <i>Profile of Mood States (Total)</i>   | 34.60 (5.90)             | 22.07 (5.68)             | 0.10 |
| <i>Psychological Well-being Scale</i>   | 76.64 (2.23)             | 82.04 (2.24)             | 0.11 |
| <b>Health related Quality of Life (SF-36)</b>   |                          |                          |      |
| <i>Mental Health Component Score</i>  | 65.25 (3.62)             | 69.46 (3.66)             | 0.42 |
| <i>Physical Health Component Score</i>  | 71.48 (3.92)             | 67.92 (3.99)             | 0.53 |

Associations between change in AD markers and changes in measures of cognitive function and psychosocial status are summarized in Table 4 (pooled sample). Improvements in memory functioning (MFQ) at 3 months were positively correlated with rises in TA, and at both 3 and 6 months with increased TL and A $\beta$  levels (A $\beta$ <sub>40</sub>, A $\beta$ <sub>42</sub>); improvements in the Frequency of Forgetfulness subscale showed the strongest associations with these biomarkers (adjusted  $r_s$  = 0.3–0.4,  $p_s$  < 0.03). Likewise, memory concerns at 6 months were inversely associated with both TL and A $\beta$  levels (A $\beta$ <sub>40</sub>, A $\beta$ <sub>42</sub>; adjusted  $r_s$  = –0.3–0.4). Increases in TA and A $\beta$ <sub>38</sub> levels were associated with improvements in cognitive performance (TMT-A and -B, respectively) at 3 months, with these correlations further strengthened (TMT-A/B) at 6 months (adjusted  $r_s$  = 0.3–0.5).

Biomarker changes were also significantly associated with alterations in psychosocial status and QOL. Longer TL at 3 months was significantly associ-

ated with gains in multiple domains of QOL at 3, although not at 6 months ( $r_s$  = 0.3–0.4). Increases in TA and A $\beta$  levels were positively associated with improvements in perceived stress and mood at 3 months ( $r_s$  = 0.4–0.5), although these associations were attenuated at 6 months. Increases in plasma A $\beta$  levels were also strongly related to positive changes in measures of stress, psychological well-being, and multiple domains of QOL (adjusted  $r_s$  = 0.3–0.6) at 3 months; associations of A $\beta$  levels with emotional well-being and QOL-physical health component were maintained or further strengthened at 6 months (Table 4). Improvements in sleep quality were likewise strongly and positively related to rising plasma A $\beta$  levels at both 3 and 6 months ( $r_s$  = 0.3–0.5). Although improvements in 3 of the 4 constituent domains of QOL-MH were significantly associated with increases in A $\beta$  levels (A $\beta$ <sub>40</sub>, A $\beta$ <sub>42</sub>, A $\beta$ <sub>42/40</sub> ratio, adjusted  $r_s$  = 0.3–0.4), change in QOL-MH overall was not ( $p_s$  > 0.1).



Table 3a

Change over time in cognitive function, psychological status, sleep, and quality of life in older adults with subjective cognitive decline randomized to a 12 week Kirtan Kriya (KK) meditation program or a 12 week music listening program

| Outcome Measures   | Change from Baseline (KK meditation) |         |                              | Change from Baseline (Music Listening) |       |                              | $p$    | $p^{\dagger}$ | $p^{\ddagger}$ |
|--|--------------------------------------|---------|------------------------------|--|-------|------------------------------|--------|---------------|----------------|
|  | 3 months<br><i>Mean (SE)</i>         | $p$     | 6 months<br><i>Mean (SE)</i> | 3 months<br><i>Mean (SE)</i>           | $p$   | 6 months<br><i>Mean (SE)</i> |        |               |                |
| <b>Perceived Memory Function</b>   |                                      |         |                              |  |       |                              |        |               |                |
| <i>Memory Functioning Questionnaire</i>  |                                      |         |                              |  |       |                              |        |               |                |
| <i>Total</i>   | 29.50 (10.34)                        | 0.01    | 33.40 (9.15)                 | 25.44 (8.65)                           | 0.007 | 30.21 (11.60)                | 0.02   |               |                |
| Frequency of Forgetfulness   | 23.15 (7.27)                         | 0.005   | 26.90 (6.58)                 | 13.96 (5.32)                           | 0.015 | 16.95 (6.29)                 | 0.01   |               |                |
| Seriousness of Forgetting  | 6.75 (2.94)                          | 0.03    | 3.80 (0.99)                  | 9.84 (3.50)                            | 0.01  | 6.75 (5.27)                  | 0.21   |               |                |
| Retrospective Memory Functioning   | 2.25 (0.79)                          | 0.010   | 3.85 (0.82)                  | 2.64 (1.14)                            | 0.03  | 4.13 (0.96)                  | 0.0003 |               |                |
| <b>Executive Function, Information Processing/Psychomotor Speed, Attention, Working Memory</b> |                                      |         |                              |  |       |                              |        |               |                |
| <i>Digit Symbol Substitution Test</i>  | 2.25 (1.12)                          | 0.05    | 5.45 (1.16)                  | 3.04 (1.00)                            | 0.006 | 4.63 (1.04)                  | 0.0002 |               |                |
| <i>Trail-making Test (TMT)<sup>†</sup></i>   |                                      |         |                              |  |       |                              |        |               |                |
| TMT-A  | -3.21 (2.19)                         | 0.16    | -6.21 (2.04)                 | -5.52 (2.50)                           | 0.04  | -5.96 (1.97)                 | 0.006  |               |                |
| TMT-B  | -14.70 (6.67)                        | 0.05    | -23.10 (7.72)                | -21.28 (6.68)                          | 0.004 | -22.54 (6.65)                | 0.003  |               |                |
| <b>Memory Concerns at 6 Months (%)<sup>†</sup></b>   |                                      |         |                              |  |       |                              |        |               |                |
| More concerned   |                                      |         | (%)                          |  |       | (%)                          |        |               |                |
| Same amount of concern   |                                      |         | 0.00%                        |  |       | 8.33%                        |        |               |                |
| Less/Much less concerned   |                                      |         | 47.37%                       |  |       | 41.67%                       |        |               |                |
|  |                                      |         | 52.63%                       |  |       | 50.00%                       |        |               |                |
| <b>Stress, mood, well-being and sleep quality</b>  |                                      |         |                              |  |       |                              |        |               |                |
| <i>Perceived Stress Scale</i>  | -4.10 (1.39)                         | 0.0008  | -6.15 (1.27)                 | -2.72 (1.45)                           | 0.07  | -3.29 (1.47)                 | 0.04   |               | (*)            |
| <i>Profile of Mood States</i>  |                                      |         |                              |  |       |                              |        |               |                |
| <i>Total</i>   | -24.90 (5.14)                        | 0.0001  | -34.00 (5.08)                | -13.80 (6.03)                          | 0.03  | -19.12 (5.00)                | 0.001  | *             | (*)            |
| Tension/Anxiety  | -4.45 (1.62)                         | 0.008   | -7.10 (1.31)                 | -2.16 (1.06)                           | 0.06  | -3.33 (1.06)                 | 0.004  | (*)           | *              |
| Confusion  | -4.80 (1.05)                         | 0.0002  | -5.75 (1.02)                 | -2.24 (1.72)                           | 0.21  | -3.67 (0.85)                 | 0.0002 | (*)           | (*)            |
| Depression   | -4.30 (1.33)                         | 0.004   | -6.40 (1.56)                 | -2.28 (0.92)                           | 0.02  | -3.04 (1.22)                 | 0.02   | (*)           | (*)            |
| Anger/Hostility  | -4.50 (1.51)                         | 0.008   | -5.50 (1.47)                 | -2.48 (1.20)                           | 0.05  | -2.87 (1.06)                 | 0.01   |               | (*)            |
| Vigor  | 1.59 (1.30)                          | 0.22    | 3.41 (1.04)                  | 1.52 (1.09)                            | 0.18  | 2.17 (1.19)                  | 0.09   |               | (*)            |
| Fatigue  | -5.45 (0.97)                         | 0.00002 | -5.85 (1.10)                 | -3.12 (1.38)                           | 0.03  | -4.04 (1.29)                 | 0.005  | *             | *              |
| <i>Psychological Well-being Scale</i>  | 7.10 (1.56)                          | 0.0002  | 7.65 (2.02)                  | -2.24 (1.13)                           | 0.06  | 2.54 (1.69)                  | 0.15   |               |                |
| <i>Pittsburgh Sleep Quality Index</i>  |                                      |         |                              |  |       |                              |        |               |                |
| <i>Total score</i>   | -1.47 (0.52)                         | 0.001   | -1.33 (0.69)                 | -1.20 (0.45)                           | 0.01  | -1.31 (0.46)                 | 0.009  |               |                |
| <b>Health related Quality of Life (SF-36)</b>  |                                      |         |                              |  |       |                              |        |               |                |
| <i>Mental Health Component</i>   | 8.00 (3.71)                          | 0.05    | 10.56 (3.70)                 | 4.92 (3.65)                            | 0.19  | 6.82 (3.28)                  | 0.05   | (*)           | (*)            |
| <i>Physical Health Component</i>   | 3.65 (4.50)                          | 0.40    | 3.00 (3.41)                  | 2.80 (3.55)                            | 0.44  | 1.90 (3.13)                  | 0.55   |               |                |

Repeated measures ANOVA; <sup>†</sup>Between group difference, 3 months; <sup>‡</sup>Between group difference, 6 months; \* $p < 0.05$ ; ( $^{\circ}$ ) $p < 0.1$ . <sup>†</sup>Calculated from participant responses to the 5-point Likert scale question 'How concerned are you about your memory compared to when you began the study?' SE, standard error.

Table 3b  
Change over time in telomere length, telomerase activity and plasma Aβ levels in older adults with subjective cognitive decline randomized to a 12-week Kirtan Kriya meditation (KK) or a 12-week music listening (ML) program

| Outcome Measures                    | Change from Baseline (KK)    |                       |      | Change from Baseline (ML)    |                       |      | <i>p</i> <sup>‡</sup> |
|-------------------------------------|------------------------------|-----------------------|------|------------------------------|-----------------------|------|-----------------------|
|                                     | 3 months<br><i>Mean (SE)</i> | <i>p</i> <sup>*</sup> | ES   | 3 months<br><i>Mean (SE)</i> | <i>p</i> <sup>*</sup> | ES   |                       |
| Telomere length (absolute mean, kb) | 0.29 (2.23)                  | 0.38                  | 0.27 | −2.25 (2.50)                 | 0.20                  | 0.10 | 0.32                  |
| Telomerase activity (TSR8 copies)   | 1048.88 (2733.45)            | 0.71                  | 0.10 | 1218.34 (1448.81)            | 0.12                  | 0.28 | 0.87                  |
| Plasma Aβ (pg/ml)                   |                              |                       |      |                              |                       |      |                       |
| Aβ38                                | 3.33 (13.32)                 | 0.81                  | 0.01 | 1.26 (9.08)                  | 0.57                  | 0.01 | 0.64                  |
| Aβ40                                | 2.04 (5.23)                  | 0.71                  | 0.09 | −14.13 (7.16)                | 0.06                  | 0.27 | 0.04                  |
| Aβ42                                | 0.42 (1.74)                  | 0.81                  | 0.01 | 0.41 (0.41)                  | 0.63                  | 0.04 | 0.77                  |
| Ratio Aβ42/Aβ40                     | 0.001 (0.01)                 | 0.33                  | 0.02 | 0.004 (0.001)                | 0.01                  | 0.14 | 0.098                 |

\*Repeated measures ANOVA; ‡Between group difference at 3 months (*P*'s and effect sizes calculated on log-transformed values, adjusted for age and baseline values). ES, effect size; SE, standard error.

Table 3c  
Change over time in telomere length and telomerase activity in older adults with subjective cognitive decline randomized to one of two simple 12-week mind-body programs: Modifying influence of baseline values

| Outcome Measures                           | Change from Baseline to 3 months  |                       |
|--|-----------------------------------|-----------------------|
|  | Mean (SE)                         | <i>p</i> <sup>*</sup> |
| <i>Telomere length (absolute mean, kb)</i> |                                   |                       |
| ≤50th Centile at baseline                  | 3.11 (1.54)                       | 0.05                  |
| >50th Centile at baseline                  | −5.17 (2.96)                      | 0.095                 |
|  | <i>p</i> for interaction = 0.0004 |                       |
| <i>Telomerase activity (TSR8 copies)</i>   |                                   |                       |
| ≤50th Centile at baseline                  | 1745.87 (973.33)                  | 0.01                  |
| >50th Centile at baseline                  | 318.25 (3181.64)                  | 0.10                  |
|  | <i>p</i> for interaction = 0.006  |                       |

\*Repeated measures ANOVA; 3 months, age-adjusted (*p* calculated on log-transformed values).

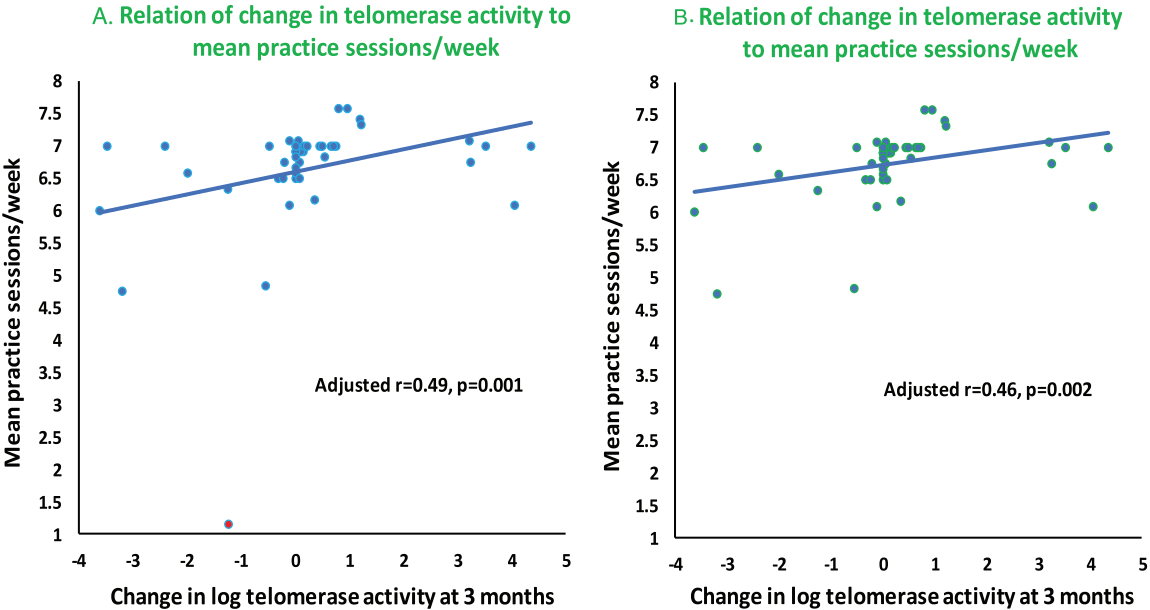


Fig. 1AB. Correlation between change in telomerase activity to mean practice sessions at 3 months with (A) and without (B) outlier included.

Table 4

Relation of changes over time in psychosocial status, quality of life, and cognitive function to those in blood biomarkers in older adults with subjective cognitive decline

| Change from baseline                    | Change over time at 3 months |                     |                     |                     |                    |                   |
|---|------------------------------|---------------------|---------------------|---------------------|--------------------|-------------------|
|   | Telomere Length              | Telomerase Activity | Aβ38                | Aβ40                | Aβ42               | Aβ42/Aβ40 ratio   |
| <b>At 3 months</b>                      |                              |                     |                     |                     |                    |                   |
| <b>Memory and Cognitive Performance</b> |                              |                     |                     |                     |                    |                   |
| Memory Functioning Questionnaire        |                              |                     |                     |                     |                    |                   |
| Total                                   | 0.34*                        |                     |                     | 0.29(*)             | 0.25(*)            |                   |
| Retrospective Memory                    |                              | 0.31*               |                     |                     |                    |                   |
| Frequency of Forgetfulness              | 0.41***                      |                     |                     | 0.43***             | 0.31*              |                   |
| Seriousness of Forgetting               |                              |                     |                     |                     |                    |                   |
| Mnemonic use                            |                              | −0.25(*)            |                     |                     |                    |                   |
| Trail-making Test-A                     |                              | −0.54*              | −0.28(*)            |                     |                    |                   |
| Trail-making Test-B                     |                              |                     | −0.40***            |                     |                    |                   |
| Digit Symbol Substitution Test          |                              | 0.26(*)             |                     |                     |                    |                   |
| <b>Mood, stress, sleep and QOL</b>      |                              |                     |                     |                     |                    |                   |
| Mood (POMS) Total score                 |                              | −0.53*              |                     | −0.41***            |                    |                   |
| Perceived stress (PSS)                  |                              | −0.29(*)            |                     | −0.32**             | −0.29*             |                   |
| Emotional well-being (PWBS)             |                              |                     |                     | 0.32**              |                    |                   |
| Sleep quality (PSQI) Total Score        |                              |                     |                     | −0.54 <sup>‡‡</sup> | −0.33*             | −0.48*            |
| Sleep Latency                           |                              |                     | −0.31*              | −0.29(*)            | −0.36**            |                   |
| Sleep quality                           |                              |                     | −0.46***            | −0.25(*)            | −0.35**            |                   |
| Sleep hours                             |                              |                     |                     | 0.26(*)             |                    |                   |
| Health-related OOL (SF-36)              |                              |                     |                     |                     |                    |                   |
| Mental Health Component                 | 0.38***                      |                     |                     | 0.32*               |                    |                   |
| Role emotional                          | 0.29(*)                      |                     |                     |                     |                    |                   |
| Emotion wellbeing                       | 0.40***                      |                     |                     | 0.36**              |                    |                   |
| Social function                         | 0.28(*)                      |                     |                     | 0.31*               | 0.26(*)            |                   |
| Energy                                  | 0.32*                        |                     | 0.40 <sup>‡</sup>   | 0.43*               | 0.55 <sup>‡‡</sup> | 0.35**            |
| Physical Health Component               | 0.33*                        | 0.26(*)             | 0.41 <sup>‡</sup>   |                     | 0.42 <sup>‡</sup>  | 0.28(*)           |
| General Health                          |                              |                     | 0.33*               |                     | 0.41***            | 0.31*             |
| Pain                                    |                              |                     | 0.26(*)             |                     | 0.27(*)            | 0.26(*)           |
| Physical function                       | 0.26(*)                      | 0.32*               |                     |                     |                    |                   |
| Role physical                           | 0.30*                        |                     |                     |                     | 0.34*              | 0.30*             |
| <b>At 6 months</b>                      |                              |                     |                     |                     |                    |                   |
| <b>Memory and Cognitive Performance</b> |                              |                     |                     |                     |                    |                   |
| Memory Functioning Questionnaire        | 0.42***                      |                     |                     | 0.37***             | 0.28(*)            |                   |
| Total                                   |                              |                     |                     |                     |                    |                   |
| Retrospective Memory                    |                              |                     |                     |                     |                    |                   |
| Frequency of Forgetfulness              | 0.41 <sup>‡</sup>            |                     |                     | 0.43***             | 0.30(*)            |                   |
| Seriousness of Forgetting               | 0.31*                        |                     |                     |                     |                    |                   |
| Mnemonic use                            |                              |                     |                     |                     |                    |                   |
| Trail-making Test-A                     |                              | −0.54*              | −0.28(*)            |                     |                    |                   |
| Trail-making Test-B                     |                              | −0.30*              | −0.39***            |                     |                    |                   |
| Digit Symbol Substitution Test          | 0.26(*)                      |                     |                     |                     |                    |                   |
| Memory concerns at 6 mos¥               | −0.33*                       |                     |                     | −0.41 <sup>‡‡</sup> | −0.29(*)           |                   |
| <b>Mood, stress, sleep and QOL</b>      |                              |                     |                     |                     |                    |                   |
| Mood (POMS)                             |                              |                     |                     | −0.29(*)            |                    | −0.27(*)          |
| Perceived stress (PSS)                  |                              | −0.43**             |                     |                     |                    |                   |
| Emotional well-being (PWBS)             |                              |                     |                     | 0.30*               | 0.35**             |                   |
| Sleep quality (PSQI)                    |                              |                     | −0.44***            | −0.39***            | −0.35**            |                   |
| Sleep Latency                           |                              |                     | −0.36**             | −0.31*              | −0.34*             |                   |
| Sleep quality                           | 0.27(*)                      |                     | −0.54 <sup>‡‡</sup> | −0.39***            | −0.48 <sup>‡</sup> |                   |
| Sleep meds                              | −0.31*                       |                     |                     | −0.42**             |                    | −0.30*            |
| Health-related OOL (SF-36)              |                              |                     |                     |                     |                    |                   |
| Mental Health Component                 |                              |                     |                     |                     |                    |                   |
| Physical Health Component               |                              |                     | 0.51 <sup>‡</sup>   |                     | 0.50 <sup>‡</sup>  | 0.38 <sup>‡</sup> |

(\*) $p < 0.1$ ; \* $p < 0.05$ ; \*\* $p < 0.025$ ; \*\*\* $p < 0.01$ ; <sup>‡</sup> $p < 0.001$ ; <sup>‡‡</sup> $p < 0.0001$ . ¥ Scored on a 5 point Likert scale from 1(much less concerned) to 5 (much more concerned). POMS, Profile of Mood States; PSQI, Pittsburgh Sleep Quality Index; PSS, Perceived Stress Scale; PWBS, Psychological Well-being Scale; QOL, Quality of Life.

As illustrated in part in Fig. 2A and B, the associations of increased A $\beta$  levels to improvements in memory function, sleep quality, mood, and QOL at both 3 and 6 months appeared more pronounced in the KK than in the ML group ( $r_s = 0.4\text{--}0.7$  versus  $0.0\text{--}0.1$ , respectively;  $p_s$  for interaction =  $0.025\text{--}0.095$ ). Surprisingly, the ML group also showed *inverse* associations between TL and improvements in both QOL-MH at 3 months and memory functioning at 6 months, as well as between TA and improvements in cognitive performance (TMT) at both 3 and 6 months ( $r_s = 0.4\text{--}0.5$ ,  $p_s < 0.04$ ).

Neither adjustment for medication or physical activity change nor exclusion of participants who changed medication ( $n=4$ ) or those who changed their physical activity routine ( $n=5$ ) in the course of the study altered the above findings. Similarly, restriction of the sample to those at most risk for cognitive decline yielded similar findings. In addition, we found no evidence for a modifying effect of treatment expectancy, depression, age, obesity, or sex on change over time in the biomarkers assessed.

## DISCUSSION

In this exploratory study, the KK group demonstrated significantly greater increases in plasma A $\beta_{40}$  levels than did the ML group, and a corresponding trend toward lower increases in the A $\beta_{42/40}$  ratio. Changes over time in both TL and TA varied significantly by baseline values, with significantly greater increases in both markers among participants with respective baseline values under the 50th percentile ( $p_s$  for interaction  $<0.006$ ). Both the KK and ML groups showed increases in TA, although changes were significant only among those above the 33rd percentile in practice adherence; TA was also significantly and positively correlated with practice adherence, despite the relatively limited variation in the latter. Both groups showed significant improvements in memory and cognitive function, as well as in measures of psychosocial status at 3 and 6 months. Improvements in stress, well-being, mood, and QOL-mental health were greater in the KK versus ML group, consistent with findings reported previously in the full sample [58]. Increases in plasma A $\beta$  levels over time were significantly correlated with gains in memory and cognitive function, and with improvements in mood, stress, sleep, and QOL at both 3 and 6 months; these relationships were particularly

pronounced in the KK group. Increases in TL and TA were also significantly associated with improvements in certain measures of psychosocial status and cognitive function.

### *Change in A $\beta$ levels over time*

To our knowledge, this is the first study regarding the effects of mind-body interventions on A $\beta$  levels in adults with or at risk for cognitive impairment. Prior studies of mind-body interventions and A $\beta$  levels in other populations are likewise sparse, to date restricted to one controlled trial, a pilot study of healthy women invited to attend a four-day vacation resort. In this pilot RCT, 64 meditation-naïve participants were randomized to either a yoga and meditation training or vacation only arm [59]; both groups showed significant post-intervention declines in plasma A $\beta_{40}$  levels and corresponding increases in A $\beta_{42/40}$  ratio, comparable to changes observed in the music (but not the KK) group in the current trial. Although physical activity has been linked to significant alternations in plasma A $\beta$  levels in cross-sectional studies [30], a recent pilot RCT of aerobic exercise versus stretching in 29 adults with MCI did not indicate significant changes in plasma A $\beta_{40}$  or A $\beta_{42}$  concentrations, although exercise participants showed (non-significantly) lower increases in A $\beta_{42}$  than did controls, and demonstrated multiple improvements in cognitive and other outcomes [60]. Reasons for the discrepant findings are unclear, but may reflect differences in the study population, nature and length of the interventions, timing of assessments, assay procedures, and other design and methodological issues.

While trials regarding the influence of non-pharmacologic interventions on plasma A $\beta$  levels remain few, plasma A $\beta$  peptides have been used to monitor the effects of anti-amyloid drugs and other pharmaceutical treatments for MCI and dementia. For example, in clinical trials of monoclonal antibodies to A $\beta$  designed to enhance A $\beta$  clearance and/or reduce A $\beta$  neurotoxicity (e.g., BAN2401 [61], solanezumab [62–65]), treatments increased plasma A $\beta_{40}$  [61–65] and A $\beta_{42}$  [62–65] in a dose-dependent manner in patients with mild to moderate AD. Trials of simvastatin yielded similar findings [66]. Consistent with findings of previous studies in adults with MCI and AD [66, 67], change in A $\beta$  levels were significantly intercorrelated in this study.

### Relation of change in plasma A $\beta$ to improvements in cognition and related outcomes

The current pilot study is also, to our knowledge, the first trial of any non-pharmacologic intervention to examine the relation of change in plasma A $\beta$  levels to improvements in cognition, mood, sleep, or related outcomes, and among the first studies of any intervention to assess these potential associations. The significant, positive association observed between increasing plasma A $\beta$  levels

and improvements in both subjective and objective measures of cognition may reflect a functional relationship, paralleling those documented between brain A $\beta$  burden and memory complaints [68, 69] and/or cognitive performance [70–73] in cross-sectional studies of non-demented older adults. Although trials of anti-amyloid and other drug treatments for cognitive impairment have not generally documented parallel improvements in cognition or other relevant functional endpoints, recent clinical studies suggest that some interventions which raise plasma A $\beta$ ,

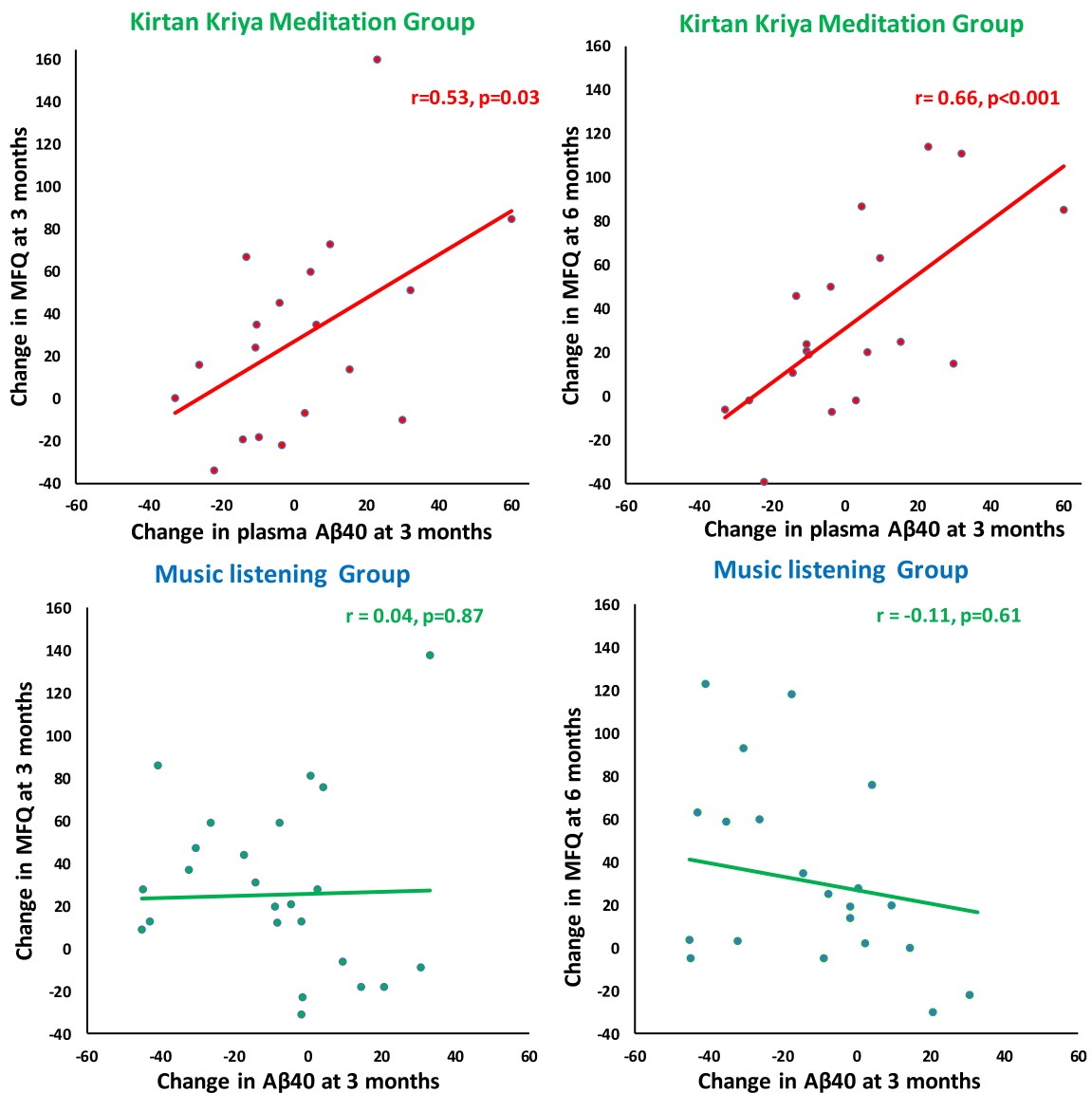


Fig. 2A. Relation of improvement in memory function at 3 and 6 months to increases in plasma A $\beta$ 40 at 3 months in older adults with SCD randomized to a 12-week Kirtan Kriya or music listening program. MFQ, Memory Functioning Questionnaire (higher scores reflect better memory function).  $p$ s for interaction = 0.10 and 0.026 for 3 and 6 months, respectively.

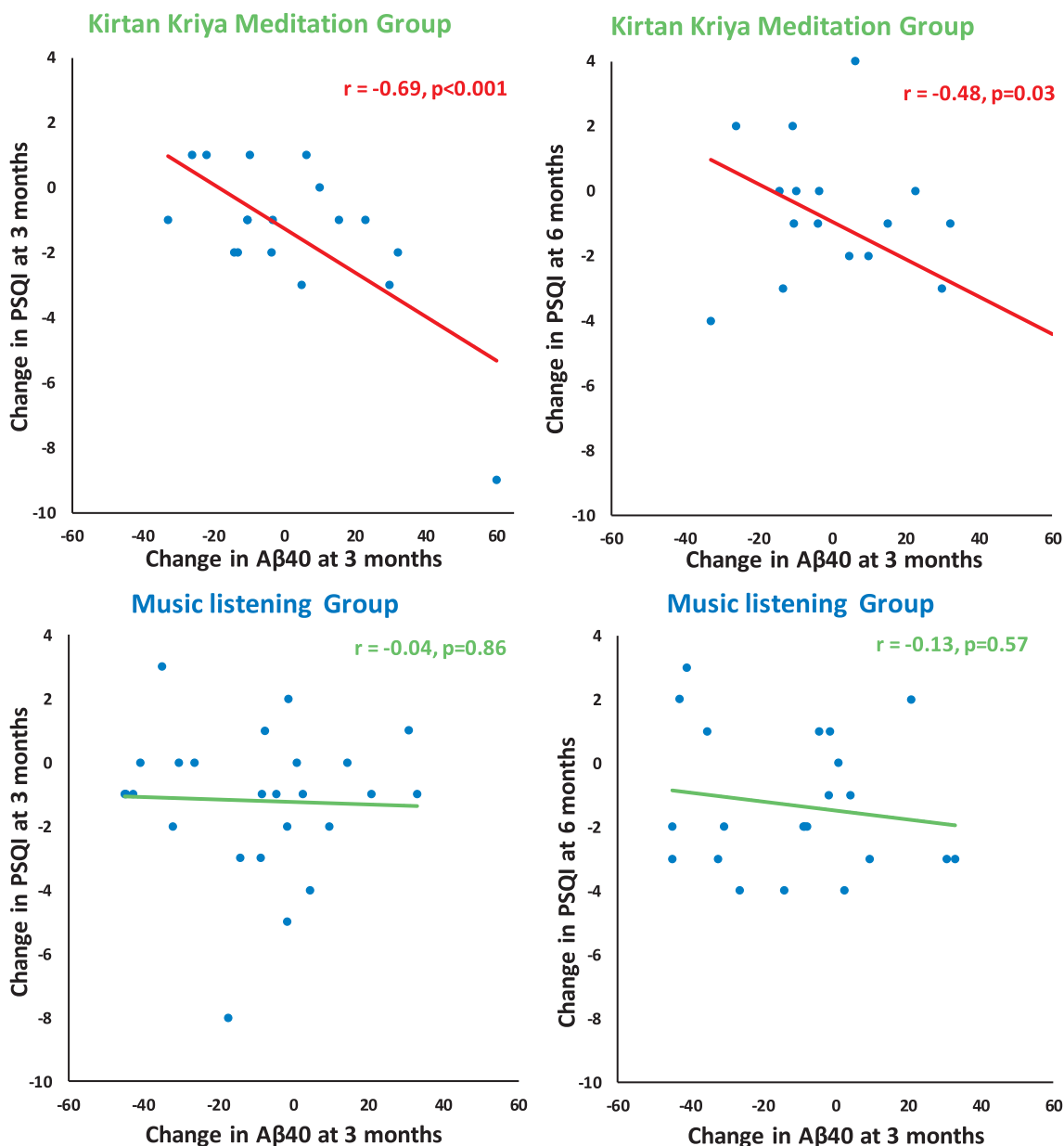


Fig. 2B. Relation of reduction in sleep impairment at 3 and 6 months to increase in plasma Aβ<sub>40</sub> at 3 months in older adults with SCD randomized to a 12-week Kirtan Kriya or music listening program. PSQI, Pittsburgh Sleep Quality Inventory (higher scores reflect greater sleep impairment). *ps* for interaction = 0.025 and 0.10 for 3 and 6 months, respectively.

including certain anti-diabetic medications, may also improve cognitive outcomes. For example, in an RCT of intranasal insulin in patients with AD or MCI, the treatment group showed significant increases in plasma Aβ<sub>40</sub> and Aβ<sub>40/42</sub> ratio, along with improvements in memory, attention, and functional status; all gains were significantly greater than those receiving placebo [74]. Consistent with the inverse

correlations between psychological distress and Aβ levels observed in this study, reductions in cortisol levels in insulin treated patients were also strongly and inversely associated with increases in Aβ<sub>40</sub> levels ( $r = -0.7, p = 0.02$ ) [74]. Likewise, in a small placebo-controlled trial of the insulin-sensitizing drug, rosiglitazone in patients with MCI or mild AD, plasma Aβ levels declined over the 6-month

treatment period in the placebo-treated patients and remained stable in the rosiglitazone-treated group. Accompanying these changes, delayed memory was preserved and attention improved in the rosiglitazone group, whereas those randomized to placebo showed progressive decline in performance [75].

#### *Relation of change in plasma A $\beta$ levels to improvement in sleep and mood*

Impaired sleep is a significant predictor of cognitive decline, and of transition to MCI and dementia [26, 76–78], a relationship that may be in part mediated by a reciprocal association between sleep and A $\beta$  burden. Recent animal model studies and a growing number of clinical and observational investigations in humans suggest that sleep impairment may contribute to A $\beta$  deposition [79], possibly by reducing clearance via the glymphatic system [80–82], and that A $\beta$  deposition may, in turn, adversely influence sleep quality [79, 83]. The strong positive association between plasma A $\beta$  levels and improvement in sleep quality observed in the current study may reflect this bidirectional relationship between sleep and A $\beta$  burden and help explain the significant associations previously reported between improvements in sleep quality and those in memory function [58, 84].

Like impaired sleep, depression, anxiety, and other distressful states have been linked to increased risk for accelerated cognitive decline, the development of cognitive impairment, and progression to dementia [85–96]. Conversely, SCD has been associated with elevated risk for increased neuropsychiatric impairment [39, 97] and diminished QOL [98, 99], suggesting a bidirectional relationship between these psychosocial factors and cognitive decline [58]. Mood disorders have also been associated with altered A $\beta$  levels and disturbances in A $\beta$  metabolism [27, 100], which may in part mediate the known adverse effects of depression and other affective disorders on cognition. In support of this hypothesis, improvements in several measures of mood, stress, and QOL were significantly correlated with increases in A $\beta$  levels in this study. As noted above, rising A $\beta$  levels were, in turn, strongly and positively related to gains in memory functioning, with associations that were particularly pronounced in the KK group. Consistent with findings detailed in our previous papers [58, 84], improvements in psychosocial status were also correlated with gains in multiple measures of memory and cognitive performance in this sample.

#### *Plasma A $\beta$ : A useful clinical marker?*

The utility of plasma A $\beta$  as a prognostic factor for cognitive change remains controversial [16, 66]. Findings regarding the relation of plasma A $\beta$  levels to the subsequent development of cognitive impairment have been contradictory [16, 66, 101]. Several studies have reported lower baseline A $\beta$  levels to be associated with higher risk for incident cognitive impairment [14–16, 102, 103], while others have found higher levels to predict conversion to MCI or dementia [17, 104, 105], and still others have indicated no relation [20, 66, 106]. In a recent study of Australian elders with AD ( $n = 39$ ), amnesic MCI ( $n = 89$ ), normal cognition ( $n = 126$ ), baseline plasma A $\beta$  levels were positively associated with global cognition and hippocampal volume and negatively with white matter hyperintensities [107]. In a longitudinal investigation of older U.S. adults, a decrease in A $\beta_{40}$  or an increase in the A $\beta_{42/40}$  ratio over the 3 year follow-up period was associated with a deterioration in cognitive performance in those with MCI ( $n = 276$ ) or normal cognition ( $n = 168$ ), but not AD at baseline [108]. Similarly, in a longitudinal study of 771 Australian adults, plasma A $\beta_{42}$  declined in those with MCI and transitioning from normal cognition to MCI; plasma A $\beta_{40}$  and A $\beta_{42}$  levels at baseline were also significantly and positively correlated with neuropsychological performance, although only in those with AD [14].

Nonetheless, while the clinical significance of plasma A $\beta$  peptides is not yet well understood, findings from recent experimental and observational studies suggest plasma A $\beta$  may be a useful marker of central nervous system (CNS) amyloid burden. For example, recent experimental research in humans suggest that transport into the blood is a significant mechanism of clearance for A $\beta$  produced in the CNS [109]. In a cohort of 719 adults with and without cognitive impairment [110], investigators found moderate positive correlations between plasma and cerebrospinal fluid (CSF) levels for both A $\beta_{42}$  and A $\beta_{40}$ , as well as negative correlations between plasma A $\beta_{42}$  and neocortical amyloid deposition (measured with PET) (healthy, SCD, MCI, and AD). Similarly, a study of 771 participants from the Australian Imaging Biomarkers and Lifestyle (AIBL) study [14], documented significant inverse correlations between plasma A $\beta_{1-42}$ /A $\beta_{1-40}$  and neocortical amyloid burden in AD patients. The potential value of plasma A $\beta$  as a clinical biomarker is also highlighted by its current use in evaluating the effects

of potential AD interventions, including anti-amyloid drugs, as discussed above.

### *Telomeres and telomerase activity*

Immune cell telomeres are DNA-protein structures that function as caps protecting and stabilizing the ends of mammalian chromosomes during cell division [111, 112]. Shortened telomeres are considered a marker of cellular aging and vulnerability to apoptosis, and potentially, of cognitive and biological aging [3, 113]. Shorter TL has been associated with diabetes, cardiovascular disease, stroke, and other chronic conditions, with cardiometabolic and lifestyle-related risk factors for chronic disease [5, 31, 114], with chronic stress and depression [7, 28, 115], and with premature mortality [116]. However, the link between TL and development of cognitive impairment or dementia is still unclear [117, 118]. Some longitudinal studies have reported a link between shortened telomeres and incident MCI dementia [10] and modest associations with subsequent cognitive decline [9], while other studies have documented cross-sectional but not prospective associations with cognitive performance [12] or conversion to AD [119], or no association between TL and cognitive decline or incident cognitive impairment/dementia [5, 11, 12, 120]. In one nested case-control study, both short and long telomeres were significantly associated with conversion to MCI [8]. Interestingly, TL has likewise been both positively [121] and inversely [122] correlated with hippocampal volume.

TL and integrity are in part maintained and protected by telomerase, a naturally occurring enzyme that plays a critical role in cell survival, extending TL and promoting cell growth, and thus helping to delay or reverse cellular aging [123]. Like aging and cognitive decline, TL and TA are affected by multiple behavioral and lifestyle factors. For example, findings of a recent systematic review indicate basal TA may be reduced in chronic stress and increased in depression and certain other distressful states [29], perhaps reflecting chronic, long term versus acute (rescue) effects. Consistent with this hypothesis, there is some evidence to suggest that TA is increased under conditions of acute psychological stress [123].

In this study, while both the KK and ML groups showed nonsignificant increases in TA, increases in TA were significantly greater among those with higher versus lower practice adherence, a finding consistent with the significant, positive association

observed between practice adherence and rise in TA. Changes over time in both TL and TA varied by baseline values of these biomarkers, with significantly greater increases in both markers among participants with respective baseline values under the 50th percentile. Increases in TL and TA were significantly associated with improvements in certain measures of psychosocial status and cognition, including memory functioning, cognitive performance (TA), mood and perceived stress (TA), and multiple domains of QOL.

While, to our knowledge, this is the first study to assess the potential influence of mind-body therapies on TL and TA in adults with SCD, several RCTs of meditative practices conducted in other populations have included one or both of these measures. The latter include studies of mindfulness meditation-based interventions in breast cancer patients [124, 125] and overweight women [126], Transcendental Meditation in hypertensive African American adults [127], KK versus ML in dementia caregivers [128], qigong and meditation in adults with chronic fatigue [129], and tai chi in healthy Chinese older adults [130]. Interventions ranged from 8 weeks [124, 128] to 6 months [130]. Of these seven trials, only two included measures of TL, both studies of mindfulness-based interventions in breast cancer patients [124, 125]. In agreement with our findings, neither reported significant changes over time or significant differences between groups, although in one study, investigators noted a trend toward telomere maintenance in the intervention groups versus usual care [124]. In agreement with findings of Carlson et al. [124], increasing TL was not related to reductions in perceived stress or mood impairment in the current study, although we did find positive associations with improvements in several domains of QOL-MH.

Of the six RCTs assessing change in TA [125, 126, 128–130] or telomerase gene expression [127] with mind-body therapies, most [125, 127–130] but not all [126] have reported significant or marginally significant between-group differences over time. In contrast to our findings, the former documented greater increases in TA with mind-body programs relative to a waitlist/non-active [125, 129, 130], health education [127], or relaxation control [128]. Consistent with our results, TA was positively associated with class attendance, and with declines in anxiety and chronic (although not perceived) stress in a trial of overweight/obese women [126] and with improvements in some measures of mental health in a pilot trial in dementia caregivers [128].



### *Strengths and limitations*

Strengths of this study include the rigorous, randomized study design and the overall high adherence and retention rates in both groups. We included well-validated measures of both subjective and objective cognitive performance, as well as sleep, stress, mood, and QOL, permitting us to assess the relation of change in AD biomarkers to improvement in multiple domains of cognitive function and psychosocial status. Data on treatment expectancy also allowed adjustment for potential placebo effects. Information on change in medication use and in physical activity permitted us to assess the potential modifying influence of these factors on change in biomarkers. Blood collection was performed by experienced phlebotomists with specific expertise in challenging populations.

To ascertain presence of SCD, we used a 6-item questionnaire based on prior expert reviews and risk analyses available at the time, and eligibility criteria which included concerns regarding memory problems, a factor shown to further increase the risk for MCI and AD associated with perceived memory loss [40–42, 131], helping us to identify those at risk for cognitive decline. Moreover, certain characteristics of our study sample suggest we were successful in capturing an at-risk population. For example, mean participant baseline scores on the MFQ were comparable to those of adults with amnesic MCI [56], and substantially lower than those reported in community-based samples [57]. In addition, baseline TMT-B scores in over 40% of participants were in the range suggesting high risk for accelerated cognitive decline and conversion to MCI/dementia [55, 132]. Finally, our participants were also characterized by high prevalence of known AD risk factors.

However, this exploratory study also has several limitations. Our study sample size was relatively small, and our participants were relatively young, well-educated, motivated volunteers with SCD, potentially limiting generalizability to other populations with memory loss. While we assessed memory and cognitive function, we did not perform diagnostic cognitive testing in our sample; it is thus possible that participants included some individuals with undiagnosed MCI. We also lacked information on the APOE4 genotype, and thus were unable to evaluate the potential modifying effects of this factor on change over time in blood biomarkers. Blood samples were not available for all participants, potentially introducing bias. However, there were no baseline

differences between those with and without missing blood samples. We did not measure A $\beta$  levels, TL, or TA at 6 months, so were unable to assess potential longer term effects of KK or ML on these biomarkers.

Plasma concentrations of A $\beta$  are substantially lower than those in CSF, potentially rendering detection of these peptides challenging [133]. However, the use of the MSD electrochemiluminescence assay has been shown to perform well in detecting CSF levels of A $\beta$  in both cognitively healthy and cognitively impaired adults [134–136] and to provide superior reliability, sensitivity, recovery, accuracy, and precision in the measurement of other low volume blood biomarkers [137]. Nonetheless, observed variability in blood biomarkers was high in our sample, reducing power and limiting our ability to detect differences in these indices.

In addition, this exploratory RCT did not include a specific measure of episodic memory, a potentially important limitation. However, we did include the MFQ, a measure of subjective memory function. MFQ scores have been inversely associated with brain amyloid burden in cognitively normal older adults [68, 69, 138], and shown to differentiate non-cognitively impaired adults from those with SCD [139], suggesting that the MFQ may offer a useful measure for detecting preclinical AD. Importantly, subjective memory complaints have been shown to predict accelerated deterioration in cognitive function [42, 94], an up to 4.5 fold increased risk for progression to MCI [85, 94], and a 2–6.5 fold increased risk for AD [40, 140] after adjustment for demographics, depression, APOE4 status, and other risk factors. Recent prospective research has likewise shown SCD in clinically normal elders to remain strongly associated with cognitive performance even after adjustment for AD biomarkers [42, 141], including A $\beta$  [142]. Notably, concern regarding one's memory problems has alone been linked to significantly increased risk for accelerated cognitive decline and conversion to MCI and AD [41, 42, 131, 143], again indicating that subjective memory complaints may be a sensitive indicator of both current cognitive function and risk for subsequent cognitive decline.

Furthermore, we employed other objective measures of cognitive performance, including the TMT-A/B, a well-established measure of multiple domains of executive function (including working memory), and the DSST, a sensitive and well-validated measure of processing speed, working memory, and attention. Both instruments have also proven useful as predictors of subsequent cognitive

impairment and dementia. For example, in a recent longitudinal study of memory clinic patients with SCD, worse TMT-B scores independently predicted subsequent conversion to MCI or AD [132]; score on the TMT-B was also shown to be the best single predictor for dementia in a prospective study of Swedish MCI patients [55]. Likewise, in a large cohort study of French elders, poor performance on the DSST was a significant, independent predictor of 9-year risk for dementia [144]. Studies of non-demented older adults have also indicated executive function and processing speed may mediate changes in episodic memory, with one study reporting the DSST to account for over 98% of the variance in age-related differences in episodic memory [145].

In addition, because the study lacked a non-active control group, we could not evaluate the influence of time trends on change over time in the biomarkers measured, although the significant association observed between change in TA and practice adherence helps to increase confidence in our findings. Nonetheless, addition of a non-active control could significantly strengthen the study design and should be considered for future trials. Such a comparator could, for example, include enhanced usual care, waitlist control, or a sham intervention designed to mirror the active intervention in the non-specific (but not 'active') components (i.e., time, attention, setting, etc.), with the caveat that the challenges of designing appropriate control groups for mind-body interventions are many and warrant careful consideration [146].

Moreover, while placebo effects cannot be completely ruled out, treatment expectancies in this study were unrelated to changes in AD biomarkers or other outcomes, suggesting that such effects are unlikely to explain our findings. Furthermore, although both groups received an active intervention in this study, and the interventions were comparable in multiple non-specific components, we did, as noted above, observe a number of key between-group differences. For example, the KK group showed greater improvement in several psychosocial outcomes, greater increases in A $\beta$ <sub>40</sub> levels, and stronger associations between increases in A $\beta$  levels and improvements in memory function, sleep, mood, and QOL than did the ML group. These findings suggest that the two interventions may have differential effects on certain outcomes, and may operate in part via different mechanisms.

Although we assessed the potential modifying effects of change in physical activity, we lacked

information on change in other lifestyle factors that may have affected outcomes, including diet. In addition, the relatively narrow range of practice adherence due to the overall high rates of practice in this study limited our ability to investigate the relationships of practice to change in AD markers. While we did note significant associations between adherence and telomerase activity, these findings must be interpreted with caution and warrant replication in larger studies.

Finally, we did not exclude those with a history of depression or anxiety, or those currently under treatment for these disorders, which may have influenced our results. However, in this study, history of depression was unrelated to baseline cognitive scores or to biomarker levels; we also found no evidence for either a modifying or confounding effect of history of depression or use of antidepressant medication, suggesting these factors did not influence our findings. Depression and anxiety are both strong predictors of subsequent cognitive decline and dementia in previously cognitively intact adults [88, 92]; at least two AD risk scales for non-demented adults include depression [147]. Therefore, we consider adults with depressive symptoms to be an at-risk group that should not be excluded from intervention studies for improving cognitive function.

## Conclusions

Findings of this exploratory RCT suggest that practice of KK meditation or a simple ML program may alter plasma A $\beta$  levels, TA, and possibly, TL. Clearly, additional rigorous longitudinal research is warranted to confirm and extend these preliminary findings in a larger population of older adults with early memory loss, and to assess longer term changes in TL, TA, and plasma A $\beta$  with KK and ML. Additional research is also needed to: evaluate the association of changes in plasma A $\beta$  to those in CSF and neuroimaging measures of A $\beta$  levels, in neurological structure and function, and in additional measures of cognitive performance; and to further investigate the dynamic relationships between A $\beta$ , cognition, sleep, mood, and related outcomes.

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