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Cognitive Behavioral Therapy and Tai Chi Reverse Cellular and Genomic Markers of Inflammation in Late Life Insomnia: A Randomized Controlled Trial

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Abstract

Background—Sleep disturbance is associated with activation of systemic and cellular inflammation, as well as pro-inflammatory transcriptional profiles in circulating leukocytes. Whether treatments that target insomnia-related complaints might reverse these markers of inflammation in older adults with insomnia is not known.

Methods—In this randomized trial, 123 older adults with insomnia were randomly assigned to cognitive behavioral therapy for insomnia (CBT-I), tai chi chih (TCC), or sleep seminar education active control condition (SS) for two hour sessions weekly over 4 months with follow-up at 7- and 16-months. We measured C-reactive protein (CRP) at baseline, month 4 and 16, Toll-like receptor-4 (TLR-4)-activated monocyte production of proinflammatory cytokines at baseline, month 2, 4, 7, and 16, and genome-wide transcriptional profiling at baseline and month 4.

Results—As compared to SS active control, CBT-I reduced levels of CRP (month 4, 16, P's<0.05), monocyte production of proinflammatory cytokines (month 2 only, P<0.05), and pro-inflammatory gene expression (month 4, P<0.01). TCC marginally reduced CRP (month 4, P=0.06), and significantly reduced monocyte production of proinflammatory cytokines (month 2, 4, 7, 16, all P's<0.05) and proinflammatory gene expression (month 4, P<0.001). In CBT and

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TCC, TELIS promoter-based bioinformatics analyses indicated reduced activity of nuclear factor (NF)-κB and AP1.

Conclusions—Among older adults with insomnia, CBT-I reduced systemic inflammation, TCC reduced cellular inflammatory responses, and both treatments reduced expression of genes encoding proinflammatory mediators. The findings provide an evidence-based molecular framework to understand the potential salutary effects of insomnia treatment on inflammation, with implications for inflammatory disease risk.

Keywords

Insomnia; Inflammation; Cognitive behavioral therapy; Tai Chi; Gene expression; Aging

INTRODUCTION

Insomnia, diagnosed by difficulties in initiating sleep, frequent awakenings, or inability to return to sleep, which are associated with daytime impairments (1), occurs in over 15% of older adults (2). Given that poor sleep prospectively predicts depression (3–5), chronic disease risk (6), and mortality (7), increasing attention has focused on the association between sleep disturbance and inflammation (8). Activation of cellular signals that initiate the production of inflammatory cytokines and markers of systemic inflammation (i.e., C-reactive protein, CRP) are associated with risk of depression (9), and a wide spectrum of medical conditions (10–14).

The causal relationship between insomnia and inflammation remains unclear. Exogenously triggered activation of inflammation induces depressive symptoms (15, 16) and also alters sleep in humans (17, 18). Conversely, insomnia is associated with elevated levels of proinflammatory cytokines (8, 19). Indeed, decreases in sleep duration are prospectively associated with increases in CRP (20), and experimental sleep disruption induces increases in CRP)(21), increases in cellular inflammation (22, 23), and increases in the expression of inflammatory response genes (23) via activation of the transcription factor, nuclear factor (NF)-κB (24). In the present study, we sought to determine whether two experimental interventions that improve insomnia symptoms (25), might reduce systemic and cellular markers of inflammation and reverse inflammatory gene expression and activation of transcriptional signaling.

In persons experiencing significant life adversity, cognitive behavioral stress management, as well as meditation, can at least partially reverse the pattern of leukocyte proinflammatory transcriptional alterations associated with stress (26–28). However, these small randomized controlled trials have not targeted patients with insomnia, nor comprehensively captured a vertically integrated assessment of inflammation including systemic levels (e.g., CRP), upstream cellular production of proinflammatory cytokines (e.g, Toll-like receptor (TLR)-4 activation of monocytic production of proinflammatory cytokines), and gene expression with promoter based bioinformatics analyses of several specific transcription factors (TF). TLR-4 activation mediates innate immune responses to common pathogens (29), and aberrant increases of TLR-4 activity are linked to inflammatory (30) and cardiovascular disease (31).

Cognitive behavioral therapy for insomnia (CBT-I), a multi-component behavioral intervention that provides sleep education, stimulus control (strengthening associations between bed and sleep), and therapy for anxiety-provoking beliefs about sleep, primarily targets sleep behaviors with effects on arousal mechanisms. CBT is an effective treatment for insomnia in older adults (25, 32) and adults (33), with an efficacy that is better sustained than pharmacotherapy (34). As a comparison to CBT-I, Tai Chi Chih (TCC), a westernized version of Tai Chi (35–37), is thought primarily to target arousal mechanisms with secondary effects on insomnia (38–40) which in turn decreases sympathetic activation and related inflammation (41, 42). TCC improves sleep quality (43–45), and reduces inflammation in older adults (25, 46–49).

In a randomized controlled, comparative efficacy trial over 4 months with follow-up at 7 and 16 months in 123 older adults with insomnia, we previously reported that CBT and TCC were associated with improvements in sleep quality, fatigue, and depressive symptoms as compared to an active control, sleep seminar (SS) (25). In addition, remission of insomnia was associated with reduced proportion of having high CRP (>3.0 pg/ml) at month 16. SS controlled for non-specific factors (e.g., expectation, group, and attention). Given evidence linking sleep disturbance, as well as related arousal mechanisms to inflammatory dynamics (8), we hypothesize that both CBT-I and TCC, in this same sample (n=123), will reverse increases in levels of CRP, increases in TLR-4 induced activation of monocyte production of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF), and increases in pro-inflammatory gene expression programs and the specific pattern of bioinformatically inferred TF activation (i.e., increased activity of NF-κB/Rel and AP1 TFs) relative to SS. Because levels of CRP are relatively stable and changes in CRP in response to behavioral interventions (i.e., exercise) are found after year-long, not months, administration (50–52), CRP was measured at baseline, month 4 (post-intervention), and month 16. In contrast, because changes in TLR4 induced monocytic production in IL-6 and TNF have been found immediately after sleep disturbance (23), this marker was measured at baseline, month 2 (mid-intervention), and month 4, 7, and 16. Post-intervention differences in the expression of *a priori* defined pro-inflammatory gene programs and bioinformatically inferred TF activation were tested in a subsample.

METHODS

Participants

This randomized controlled trial was conducted from April 2006 to August 2011 with UCLA IRB approval. As described (25), 123 community-dwelling adults older than 55 years of age who fulfilled criteria for primary insomnia in *Diagnostic and Statistical Manual* (Fourth Edition, Text Revision)*(DSMIV-TR)*(53) and for general insomnia in the *International Classification of Sleep Disorders* (Second Edition)(54) were randomly assigned to CBT-I, TCC, or SS (2:2:1). Complete inclusion criteria are provided in Supplement.

Interventions

Each group participated in 120-minutes of class time weekly for 4 months with 7- and 16 months follow-up. CBT-I was modified to teach behavioral strategies for management of daytime activity levels and enhancement of mood (33). TCC, a movement meditation, emphasized control over arousal mechanisms, which are thought to contribute to insomnia (55, 56). Sleep Seminar (SS) provided sleep hygiene information and education about physical, medical, and psychosocial factors in relation to aging and insomnia. Supplement provides information about the treatments, acceptance, credibility, and expectation for change (25).

Outcomes

Insomnia outcomes included remission of insomnia diagnosis by DSM-IV-TR criteria using a structured interview and checklist, and improvements in patient-reported outcomes of insomnia symptom severity and daily sleep diaries (25). In this study, assessment of inflammation included three levels of analysis: systemic (i.e. CRP levels), cellular (i.e., TLR-4 activation of monocytic production of inflammatory cytokines TNF and IL-6) and genomic (i.e., gene expression and bioinformatics analyses of transcription pathways). Prior to each blood sampling, subjects were queried about recent (i.e., last month) infection, illness, or vaccination, and sampling was re-scheduled if subjects reported any one of these issues. All blood samples were non-fasting and collected between 8–10 a.m.

CRP levels were measured (25). To clarify the functional basis for altered CRP, production of proinflammatory cytokines by monocytes following ligation of the Toll-like receptor 4 (TLR4) with lipopolysaccharide (LPS) was assessed (23). To evaluate the upstream sources of cellular inflammatory cytokine expression, RNA from peripheral blood mononuclear cells (PBMC) were collected for evaluation of gene expression profiling and bioinformatic analysis in a random subsample at 4 months for comparison of CBT-I or TCC, relative to SS (n=78). Additionally, we determined whether gene expression profiles were comparable in three groups at baseline prior to intervention (n=24), given the effects of sleep on inflammatory gene expression (23). RNA was extracted (Qiagen PAXgene Blood RNA Kit; Valencia CA), and subject to genome-wide transcriptional profiling using Illumina HT-12 v4 BeadArrays following the manufacturer's standard protocol (Illumina Inc., San Diego CA). Quantilenormalized gene expression values were log2-tranformed and subject to general linear model analysis to provide maximum likelihood point estimates of differential transcript abundance across conditions, which provide maximally replicable inputs into the 2 higher-order set-based bioinformatics analyses (57–59). TELiS promoter-based bioinformatics analyses tested the hypothesis that PMBC from older adults with insomnia who were randomized to either CBT or TCC, relative to SS, would show alterations in global gene expression profiles consistent with decreased activity of the pro-inflammatory transcription factors NF-κB (assessed by prevalence of the TRANSFAC V $NFKAPPAB65_01 nucleotide motif in differentially expressing promoters) and AP-1 (V $AP1FJ_Q2). We also explored whether these treatments increased activity of Type I interferon signaling pathways (V$ISRE_01, V$IRF2_01); increased activity of the anti-inflammatory glucocorticoid receptor (GR) (V$GR_Q6), and decreased activity of CREB transcription factors involved in β-adrenergic signaling by the sympathetic nervous system

(SNS) (V$CREB_01), because sleep is implicated in the regulation of these pathways (60). The ratio of response element frequencies in the promoters of up- vs. down-regulated genes was taken as a measure of differential activity of transcription control pathways, and (log) ratios were averaged over 9 different parametric combinations of promoter length (−300, −600, and −1000 to +200 bp upstream of RefSeq-designated transcription start site) and motif detection stringency (TRANSFAC mat_sim values of .80, .90, and .95) to ensure robust results (61). To identify the primary cellular sources of differentially expressed genes, we carried out Transcript Origin Analysis (62). In both TELiS and Transcript Origin Analyses, standard errors were estimated by 2000 cycles of bootstrap resampling of residual vectors from the linear models used to estimate differential gene expression across groups (controlling for correlated expression across genes).

Because body mass index (BMI) and physical activity (63) are related to inflammation, changes were evaluated.

Sample size

For circulating markers of inflammation (i.e., CRP), mind-body treatments reduced circulating markers of inflammation with an effect size 0.91 (46, 48, 49), 40 per treatment group provides statistical power of 80% ($\alpha = 0.05$). For TLR-4 activation of monocytic production of cytokines, TCC reduced production of proinflammatory cytokines with an effect size of .92 (64); 40 per treatment group provides statistical power of greater than 80% ($\alpha = 0.05$). For the gene expression outcome, mind-body treatments altered transcriptional profiling with an effect size .98 (26, 27, 65); 15 per treatment group provides statistical power of greater than 80%. Sleep disturbance altered gene expression of IL-6 and TNF with effect sizes of 2.06 and 2.59 ($\alpha = 0.05$)(23); 5 per treatment group provides statistical power of greater than 80% for baseline comparison.

Statistical Methods

Intervention effects on CRP levels and TLR-4 activation of monocyte production of proinflammatory cytokines were tested on an intention-to-treat basis using a mixed model analysis of variance (ANOVA) approach without a covariance structure assumption given the variability in time between measures; data from all randomized participants were included with no imputation of missing data. The mixed model approach utilizes all available data and generates unbiased estimates under the assumption that data are missing completely at random (MCAR). In cases where there were any significant or trend ($P<0.10$) pairwise differences at baseline among the three groups, the model was adjusted for baseline to better assess relative differences at during and after treatment; this applied to TLR-4 activation of monocytic inflammatory cytokines production values but not CRP values. *A priori* contrasts (Least Significant Difference [LSD] test) tested group differences in CRP at month 4 and 16, whereas group differences in TLR-4 activation of monocytic production of proinflammatory cytokines were tested at month 2, 4, 7, and 16 Differentially expressed genes were identified based on 1.2 fold magnitude difference in average gene expression between treatment groups at baseline (n=24; CBT, n=8; TCC, n=8; SS, n=8) and at 4 months (n=78; CBT-I, n=31; TCC, n=32; SS, n=15). Data were available on >95% of the subjects at

all timepoints among those who completed follow-up assessments. Analyses were carried out with IBM SPSS for Windows, version 22.

RESULTS

Baseline Characteristics of the Patients

A total of 294 subjects underwent baseline assessment, 207 were eligible, and 123 completed baseline (Figure S1; Supplement). Treatment groups were comparable with regards to background characteristics (Table 1); none of these variables was included as a covariate. A total of 112 (92%) participants completed the assigned interventions (month 4), and 108 (89%) completed follow-up (month 16). Those who did not complete the intervention were younger ($t(121)=2.12$; $p<0.05$), and had higher scores on the PSQI ($t(121)=1.72$; $p=0.09$) and MDFSI ($t(121)=1.78$; $p=0.05$), whereas those who did not complete the follow-up had higher scores on the IDS-C ($t(110)=2.82$; $p=0.05$) and MDFSI ($t(110)=3.23$; $p<0.001$). Other demographic and outcome variables did not differ between the completers and noncompleters at months 4 or 16. Retention rate was similar between groups ($\chi^2(2)=3.16$; $p=0.21$). The observed pattern of missing data did not violate the MCAR assumption ($\chi^2(394)=308.7$; $p=0.68$). Average rate of session attendance was similar ($F(2,120)=0.99$; $p=0.38$), and the three interventions were perceived as similarly acceptable at baseline ($\chi^2(2)=0.58$; $p=0.74$) and month 4 ($\chi^2(2)=3.58$; $p=0.17$). Among the TCC participants, frequency of practice for >30 minutes decreased from 3.3 (SD, 2.2) days to 2.3 (SD, 2.0) days ($t(33)=3.16$; $p=0.004$) from months 4 to 16. There were no significant changes from baseline to month 4 in BMI ($F(2,90.9)=1.71$; $p=0.19$), or physical activity (i.e., metabolic equivalents per week ($F(2,104.8)=1.79$; $p = 0.17$). Only two subjects reported antidepressant medications use.

Outcome of systemic inflammation

Change in mean levels of CRP in the three intervention groups from baseline to month 4 (i.e., post-intervention) and month 16 (i.e., one year follow-up) showed an overall statistical trend (group × time: $F(2.108.1)=2.43$, $p=0.09$) (Figure 1,). As compared to SS, CBT resulted in overall lower levels of CRP ($t(105.4)=2.08$ $p=0.04$). As compared to SS, TCC tended to have lower levels of CRP, but these differences were not significant at either month 4 ($t(103.1)=1.92$ $p=0.06$) or month 16 ($t(97.2)=0.13$ $p=0.90$). Whereas CBT and TCC showed similar low levels of CRP at month 4 ($t(104.3)=0.59$ $p=0.56$), CRP levels diverged at month 16; CRP levels remained low in CBT but increased in TCC ($t(97.1)=1.77$ $p=0.08$), along with sustained insomnia remission in CBT but not TCC (25).

Outcome of cellular inflammation

Change in mean levels of TLR-4 activated monocyte production of proinflammatory cytokines in the three intervention groups from baseline to month 2 (i.e., mid-intervention), month 4 (i.e., post-intervention), month 7 (i.e., 3 month follow-up), and month 16 (i.e., one year follow-up) was statistically significant for the percentage of monocytes producing IL-6 only (group × time $F(8,91.8)=3.23$, $p<0.01$; Figure 2A). As compared to SS, TCC resulted in overall lower levels of percentage of monocytes producing IL-6 only ($t(208.3)=2.72$ $p<0.01$), with significant differences at months 2 ($t(98.0)=2.87$ $p=0.005$) and 4 ($t(97.8)=3.18$

p=0.002) but not 16 (t(125.5)=1.49 p=0.14). As compared to SS, CBT-I showed a trend for lower levels of percentage of monocytes producing IL-6 at month 2 only (t(97.3)=1.70 p=0.09), but not at months 4, 7, or 16 (all P's>0.30). As compared to CBT-I, TCC also resulted in lower levels at months 4 (t(100.3)=3.39 p=0.001), 7 (t(89.5)=2.13 p=0.04), and 16 (t(126.5)=2.38 p=0.02).

The profile of change over time between the three groups was statistically significant for the percentage of monocytes producing TNF (group × time F(8,100.2)=3.29, p<0.01; Figure 2B). As compared to SS, TCC resulted in lower levels of percentage of monocytes producing TNF (t(271.6)=3.58 p<0.001), with significant differences at months 2 (t(100.1)=3.75 p<0.001), 4 (t(97.4)=3.18 p=0.005), 7 (t(103.2)=3.17 p<0.001) and 16 (t(125.4)=2.30 p=0.03). As compared to SS, CBT-I also resulted in lower levels of percentage of monocytes producing TNF at month 2 (t(98.9)=2.10 p=0.04), but not at months 4, 7, or 16 (all p's>0.43). As compared to CBT-I, TCC resulted in lower levels of percentage of monocytes producing TNF (t(276.6)=3.33 p<0.001), with non significant differences at month 2 (t(104.6)=1.91 p<0.06), but significant differences at months 4 (t(99.8)=2.87 p=0.005), 7 (t(107.9)=3.66 p<0.001), and 16 (t(125.4)=2.34 p=0.03).

Finally, the profile of change over time between the three groups was statistically significant for the percentage of monocytes co-producing TNF and IL-6 (group × time F(8,101.2)=4.63, p<0.01; Figure 2C). As compared to SS, TCC resulted in lower levels of percentage of monocytes co-producing TNF and IL-6 (t(207.8)=3.79 p<0.001), with significant differences at months 2 (t(102.3)=3.74 p=0.001), 4 (t(100.9)=3.39 p=0.001), 7 (t(93.0)=2.56 p=0.02) and 16 (t(137.4)=2.24 p=0.03). As compared to SS, CBT-I also resulted in lower levels of percentage of monocytes co-producing TNF and IL-6 at month 2 (t(100.5)=2.16 p=0.02), but not at months 4, 7, or 16 (all p's>0.57). As compared to CBT, TCC-I resulted in lower levels of percentage of monocytes co-producing TNF and IL-6 (t(213.9)=3.97 p<0.001), with significant differences at months 2 (t(109.9)=2.16 p<0.04), 4 (t(103.2) = 3.63 p<0.001), 7 (t(97.8)=3.72 p<0.001), and 16 (t(138.2)=2.93 p<0.01).

IDS-C scores as a time varying covariate did not change the results for CRP or cellular inflammation.

Outcome of gene expression

Genome-wide transcriptional profiling was carried out using blood samples in a random subsample at month 4 (n=78), with bioinformatic evaluation of genes showing a 1.2-fold up- or down-regulation in CBT-I vs. SS, or in TCC vs. SS (differentially expressed genes listed in Supplement). For CBT-I, a total of 347 gene transcripts showed a 1.2-fold down-regulation, and a total of 191 gene transcripts showed a 1.2-fold up-regulation relative to SS. Prominent among down-regulated genes for CBT-I were transcripts in involved in inflammation (e.g., *TLR-1, TNF, REL, JUN, FOSL2, IL-6, FOSB, IFNG, JUNB, IL-8, IL-1B, PTGS2*). Among CBT-I up-regulated genes were those involved interferonand antibody responses (e.g., *CD19, MX1, ISG15, OAS2, IGLL1, IFNAR1, IFITI, OASI, IFI44L, IGS*). For TCC, a total of 202 gene transcripts showed a 1.2-fold down-regulation and 52 gene transcripts showed a 1.2-fold up-regulation relative to SS. Prominent among the TCC-down-regulated genes were transcripts involved in immunological activation and inflammation

(e.g., *IL-6, IFNGR1, CD69, FOSB, FOS, IFNG, JUNB, IL-8, IL-1B, PTGS2*). Comparisons across groups at baseline showed no difference in inflammatory gene expression.

For CBT-I vs. SS, TELiS promoter-based bioinformatic analyses of genes showed differential change in expression (Figure 3A). Results indicated reduced activity of NF-κB (mean prevalence ratio = 0.44 ± standard error = 0.09, $p < .0001$), reduced activity of AP-1 (0.75 ± 0.08, $p = .0074$), and reduced activity of CREB (0.45 ± 0.11, $p = .0042$). Results also showed non-significant trends toward upregulation of interferon-activated transcription factors (IRF-1: 2.01 ± 0.71, $p = .1141$; IRF-2: 1.66 ± 0.46, $p = .1267$). Transcript origin analyses identified genes down-regulated in CBT-I as originating primarily from monocytes ($p = .0447$) and dendritic cells ($p = .0008$) (Figure 3B). No specific leukocyte subpopulation was identified as predominantly contributing to CBT-I up-regulated genes (although B cells showed a non-significant trend).

For TCC vs. SS TELiS analyses showed results similar to those for CBT-I. Results (Figure 3A indicated a near-significant trend toward reduced activity of NF-κB (0.61 ± 0.14, $p = .0561$) and significantly reduced activity of AP-1 (0.76 ± 0.09, $p = .0221$) and CREB (0.33 ± 0.09, $p = .0009$). Results also indicated a trend toward up-regulation of GR activity (1.42 ± 0.28, $p = .0522$). As with CBT, transcript origin analyses identified genes down-regulated in TCC participants as originating primarily from monocytes ($p = .0447$) and dendritic cells ($p = .0008$) (Figure 3B). B cells were identified as predominantly contributing to CBT-I up-regulated genes ($p < .0001$).

DISCUSSION

Given mounting evidence that insomnia patients are at greater risk for depression, medical comorbidities, and mortality (8); that sleep disturbance is associated with inflammation (6, 8, 20, 21); and that inflammation can lead to increased risk of depression (9, 66), cardiovascular disease (11), diabetes mellitus (14), and certain cancers (67–69), this study is significant by examining for the first time the efficacy of insomnia treatment on inflammation. These novel results link sleep disturbance to increased levels of systemic and cellular inflammation, and to increased leukocyte expression of pro-inflammatory genes in older adults with insomnia. Indeed, CBT-I reduces insomnia symptoms (25), reduces levels of CRP, and reverses activation of molecular inflammatory signaling pathways. CBT-I-induced reduction of systemic inflammation as indexed by CRP was maintained during follow-up at 7 and 16 months, consistent with maintenance of sleep improvements (25). In contrast, TCC targets stress effector mechanisms that drive insomnia complaints, reduces TLR-4-activated monocyte production of IL-6 and TNF, and reverses activation of inflammatory signaling pathways. TCC-induced reduction of cellular inflammation was maintained during follow-up at 7 and 16 months and this reversal of cellular inflammation occurred even though improvements in sleep disturbance were not maintained in the long-term, suggesting that these changes are independent of improvements in sleep. The ultimate duration of transcriptional impact of CBT-I and TCC remains to be established. Importantly, these effects were identified from a controlled, randomized intervention trial analyzed by intent-to-treat, which was not confounded by differences in patient characteristics.

CBT-I effects on CRP may have implications for aging-related inflammatory disease, in which the maintenance of sleep improvements afforded by CBT-I appears to be critical for the persistent reduction of systemic inflammation. We have previously reported that CBT treatment was associated with a significant 50% decrease in the proportionate risk of high CRP (25), comparable to the benefit reported with vigorous physical activity (70) or weight loss (71). Moreover, TCC-induced changes in the cellular production of proinflammatory cytokines, as well as the effects of both CBT-I and TCC on proinflammatory gene expression in the basal leukocyte transcriptome, may also have significant implications for inflammatory disease risk, as well as cancer-related disease processes. Pro-inflammatory signaling has been linked breast cancer progression and recurrence (72, 73), whereas Type I interferon activity has been linked to reduced progression (74). Indeed, CBT-I, but not TCC, had the additional effect of increasing activity of interferon-responsive transcription factors, consistent with prior findings that CBT-I increases *ex vivo* production of interferon (75).

Other types of behavioral interventions including exercise and stress reduction approaches have immunomodulatory effects. Exercise, as compare to health education control, attenuates age-related increases in CRP in older individuals (50). Similarly, a meta-analyses of 34 studies, employing varying types of mind-body interventions (e.g., meditation, yoga, tai chi), showed a moderate effect to reduce CRP (49). Whereas TCC incorporates a component of physical activity, neither physical activity nor BMI changed in any of the groups. The pattern of pro-inflammatory transcriptional bias observed in this study of older adults with insomnia is similar to that reported in breast cancer survivors (26, 64), as well as in different populations who are reporting psychological distress (27, 28, 65).

Diverse behavioral and psychological challenges may activate a conserved transcriptional response due to sympathetic nervous system activation of pro-inflammatory transcription factors such as NF-κB (42). We speculate that TCC induces a relaxation response, which reduces sympathetic outflow (41) with effects on inflammation (42). In the present study, bioinformatic analyses indicated reduced activity of CREB family transcription factors in both CBT-I and TCC, which is consistent with reduced sympathetic nervous system signaling through β-adrenergic receptors. Further, we found that TCC marginally increased the expression of genes bearing GR response elements, indicating potentially increased glucocorticoid signal transduction or glucocorticoid receptor sensitivity to the antiinflammatory effects of cortisol. Finally, as support for the hypothesis that these behavioral treatments target a conserved transcriptional response, bioinformatic inferences of the specific cell types mediating the CBT-I and TCC alterations implicated the same myeloid lineage antigen presenting cells (i.e., monocytes), similar to transcriptional shifts in four other independent studies (26–28, 65). Direct evaluation of these transcription factors is necessary to confirm indirect inferences of transcription factor activity based on bioinformatics analyses.

Several limitations require consideration. Although physical activity did not change in any of the groups, TCC incorporated low levels of physical activity not found in CBT-I. Second, although expectancy for benefit was similar, participants were aware of their intervention assignment. Third, this study focused on older adults, and findings may not generalize to younger populations who have lower levels of inflammation. Fourth, women were over-

represented, and those with medical co-morbidity were excluded. Fifth, although subjects were required to have a regular sleep wake cycle, it is possible that circadian re-alignment might have contributed to effects. Sixth, this study used one set of therapists to deliver TCC intervention, and another set to deliver CBT and SS treatments; differences between therapists might have contributed to treatment effects. Finally, the point estimates of differential gene expression are not subject to individual hypothesis testing and serve only as intermediate effect size inputs into higher-order gene set-based bioinformatics analyses testing a priori hypotheses regarding shared transcription factor promoter motifs (i.e., inflammation-related NF-κB, GR, and CREB factors) and shared cellular origin (i.e., pro-inflammatory monocytes). This study was not designed or powered for discovery-based analyses of statistically reliable associations between experimental conditions and specific individual gene transcripts. A longer-term follow-up is needed to determine the persistence of effects on transcriptional dynamics.

This study demonstrates that CBT-I primarily targets sleep behaviors and reduces systemic inflammation, whereas TCC, which is thought to target stress effector mechanisms, reduces cellular inflammation. The components of CBT-I vs TCC, which drive these differential effects on inflammation, require further clarification. Both treatments reverse some of the major changes in immune system gene expression previously observed in association with sleep disturbance (23). Given the links between insomnia, depression, and inflammatory disease risk, these findings provide an evidence-based molecular framework to understand how behavioral interventions that target sleep may reduce inflammation and represent a third pillar, along with diet and physical activity, to promote health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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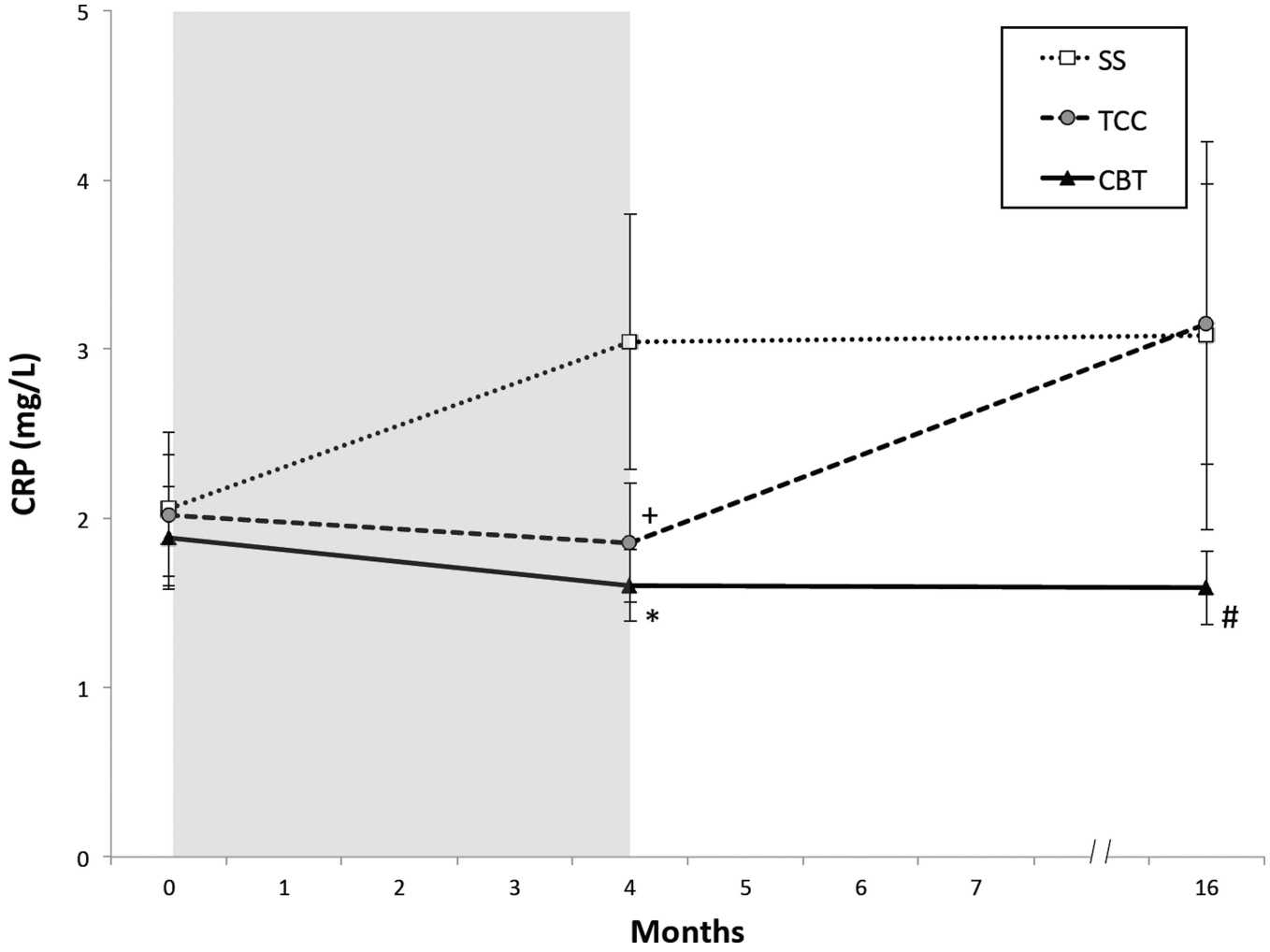

Figure 1.

Circulating Levels of CRP from Baseline to Month 16, by Treatment Group. Values are mean (SEM). Shaded area indicates period of administration of intervention following baseline assessment. Significant pairwise comparisons: *CBT vs SS P=0.02; +TCC vs SS P=0.06, #CBT vs TCC P=0.08

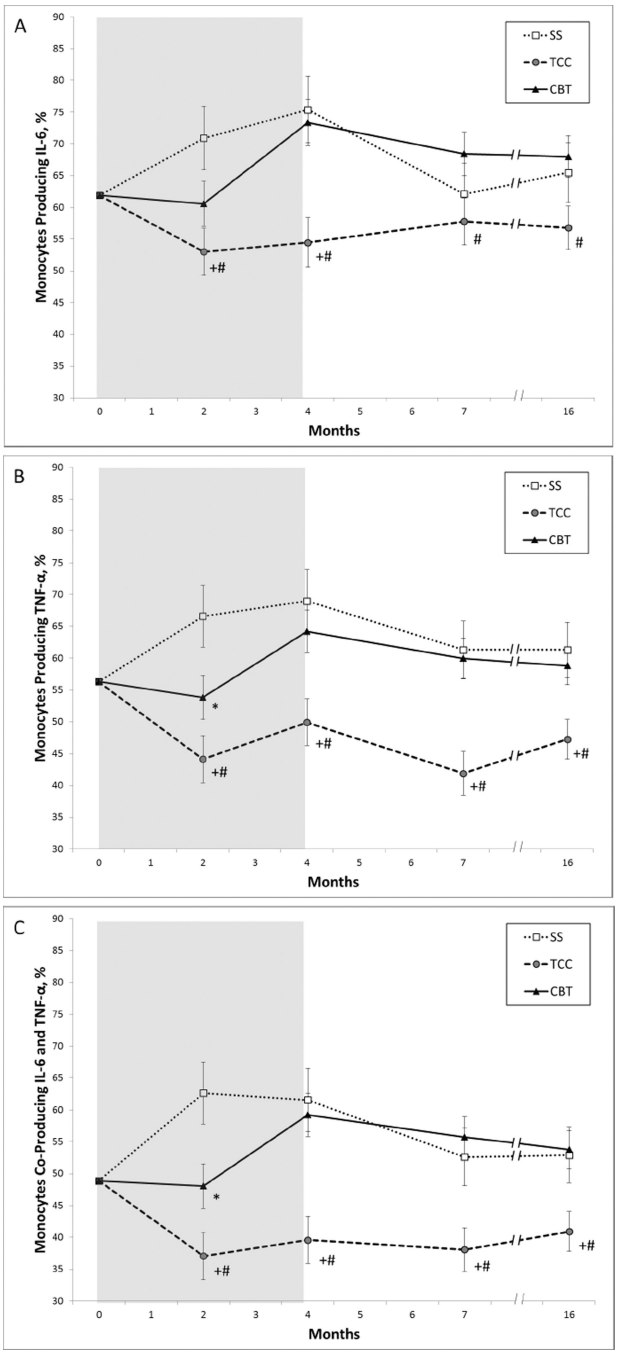

Figure 2.

Toll-like 4 Receptor Stimulated Monocytic Production from Baseline to Month 16, by Treatment Group. Values are mean (SEM) percentage of monocytes producing IL-6 (A), TNF(B); or both IL-6 and TNF(C). Shaded area indicates period of administration of intervention following baseline assessment. Significant pairwise comparisons: *CBT vs SS P<0.05; +TCC vs SS P<0.05, #CBT vs TCC P<0.05

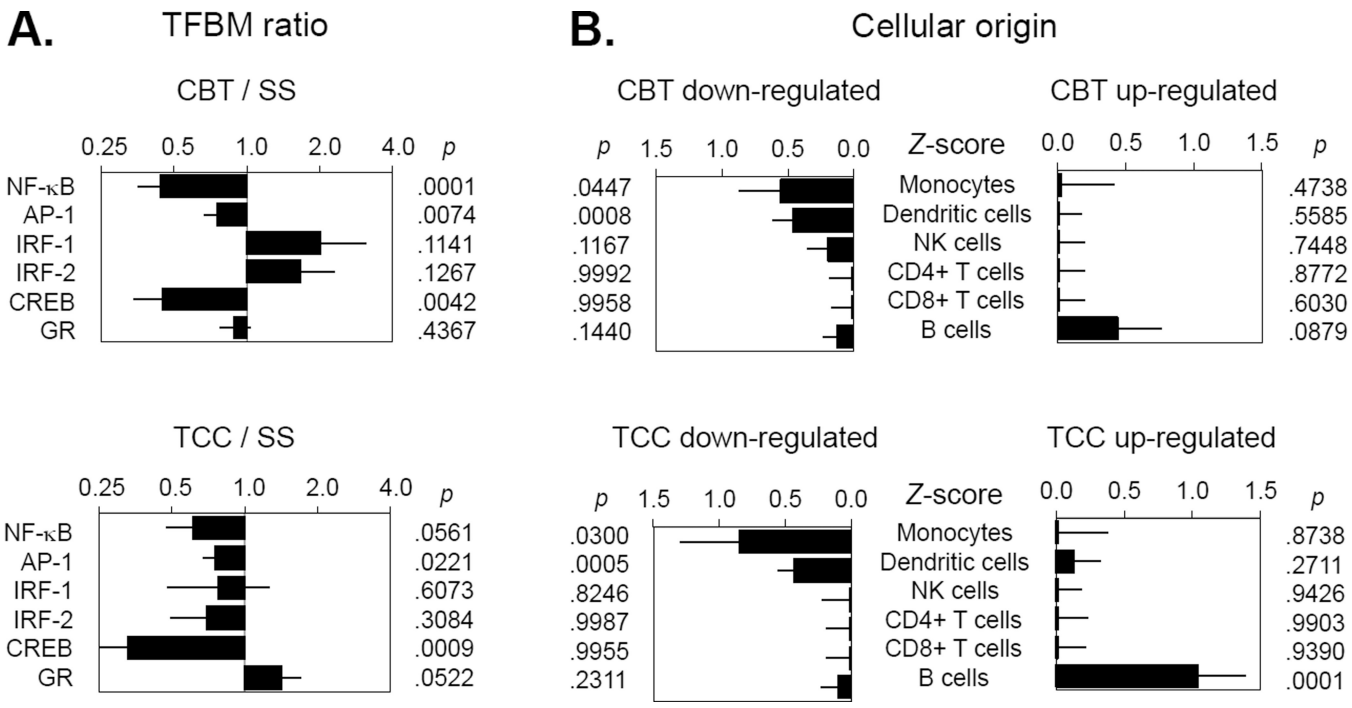

Figure 3.

Transcription factor activity as measured by TELiS promoter-based bioinformatic analyses of genes at 4 months (post-intervention), showing differential change in gene expression for comparisons of CBT vs. SS, and TCC vs. SS

Table 1

Baseline Sociodemographic and Clinical Characteristics of Participants*

Variable	CBT (N=50)	TCC (N=48)	SS (N=25)	F or χ^2; p
Age (55 to 85 years), mean (SD)	64.4 (6.1)	66.3 (7.4)	66.4 (7.7)	1.16; 0.32
Female, No. (%)	39 (78.0)	31 (64.6)	18 (72.0)	2.17; 0.34
Ethnicity, Hispanic, No. (%)	3 (6.0)	3 (6.4)	3 (12.5)	1.12; 0.57
Race, Non-white, No. (%)	6 (12.2)	7 (14.9)	4 (16.7)	0.29; 0.86
Marital status, Married, No. (%)	25 (50.0)	21 (43.8)	8 (32.0)	2.19; 0.33
Employment				
Working, No. (%)	21 (42.0)	20 (41.7)	10 (40.0)	0.03; 0.98
Work Hrs/wk, mean (SD)	13.6 (18.5)	11.7 (18.6)	11.4 (15.3)	0.18; 0.84
Education (years), mean (SD)	15.8 (1.4)	15.7 (1.5)	15.3 (1.5)	1.26; 0.29
Body mass index (kg/m^2), mean (SD)	25.4 (3.3)	26.4 (4.0)	26.3 (4.5)	0.95; 0.39
Co-morbidity				
Medical, No. (%)#	19 (39.6)	22 (48.9)	14 (56.0)	1.93; 0.38
Depression history, No. (%)	12 (24.0)	12 (25.0)	8 (32.0)	0.60; 0.74
Other psychiatric history, No. (%)	9 (18.0)	6 (12.5)	3 (12.5)	0.71; 0.70
Prior hypnotic drug usage, number (%)	12 (24.0)	7 (14.6)	2 (8.0)	3.36; 0.19

All values are mean (sd) unless noted with percentage

*Between-group differences were tested with a chi-square test for categorical variables and one-way analysis of variance for continuous variables

#Among those who reported medical co-morbidity, 95% reported cardiovascular disease