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# The Effects of a Yoga Intervention

Karen Arrant
*University of Louisiana Monroe*, arrant@ulm.edu

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Author: Karen Arrant, PhD, RN

Associate Director Baccalaureate Program

University of Louisiana Monroe

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Abstract:

Sleep disturbance and stress in postmenopausal women pose physical, mental, and emotional health hazards. Researchers examined the effect of a yoga intervention on sleep, stress, anxiety, and depression in postmenopausal women. The study employed a randomized, controlled trial with: (1) a treatment group (yoga intervention) and (2) an attention-control group (health education). All participants completed three PROMIS® – Patient-Reported Outcomes Measurement Information System – tools and collected saliva samples before and after the eight-week intervention. The PROMIS® tools measured sleep, anxiety, and depression. Salivary alpha-amylase quantified sleep; salivary cortisol measured stress; and participants self-reported hours of sleep.

Thirty-one women completed the study: 18 in the treatment group and 13 in the attention-control. The yogic intervention had a strong inverse correlation with hours of sleep ($r = -0.7717$; $p = < 0.0001$) as revealed by Pearson's correlation coefficient. We saw a moderate inverse correlation between change in depression and PROMIS pre-intervention depression scores ($r = -0.3960$; $p = 0.0303$). Hours of sleep pre-intervention compared to hours of sleep post-intervention were lower ($p < 0.0001$). Change in alpha-amylase reached statistical significance from beginning to end ($p = < 0.0001$) in the intervention group. Compared with those who received only health education, those in the yoga group experienced improved sleep. Findings are limited due to the small sample size.

The Effects of a Yoga Intervention

## Introduction

Greater than 80% of postmenopausal (PM) women participating in regular physical activity (PA) enjoy decreased stress and anxiety, while greater than 20% experience improved sleep (Alexander, Innes, Selfe, & Brown, 2013; Ancoli-Israel, 2005). However, 32% of adults do not engage in PA in early adulthood, and the percentage increases to 40% by the end of adult years (Tucker, Welk, & Beyler 2011). Health-promoting behaviors have more recently been associated with perceived stress, whereas high levels of stress may diminish the desire to be physically active (Cho, Jae, Choo, & Choo, 2014). Understanding the relationships among PA, sleep, and stress in PM women opens opportunities for targeted interventions to assure optimal aging in this population. In this study, researchers investigated these connections using a combination of objective biomarkers and subjective assessments to determine the effect of an 8-week yoga intervention on sleep and stress in a sample ($n$ = 31) of PM women.

## Background and Significance

**Sleep.** The National Sleep Foundation (NSF) (2015) recommends seven to nine hours of sleep daily for adults aged 26- 64 years and seven to eight hours for adults over age 65.  PM women experience problems with sleep, including daytime inattentiveness, disturbed nighttime sleep, and prolonged time to go to sleep (NSF, 2016; Colten, & Altevogt, 2006; Afonso et al., 2012; Hall, 2004; Banks & Dinges, 2007; Van Dongen, Maislin, Mullington, & Dinges, 2003). A number of studies suggest that encouraging PM women to participate in yoga may be a practical way of achieving lifestyle changes that will improve sleep and decrease stress (Goveas et al, 2014; Afonso et al, 2012; Booth-LaForce, Thurston, & Taylor, 2007; Banasik, Williams, Haberman, Blank, & Bendel, 2011).

Kenney (2000) noted women experience significant stress, managing multiple responsibilities such as children, aging parents, significant others, a career, and household obligations. The challenge of balancing these responsibilities may have an effect on women's emotional, mental, and physical health, thus, it affects all aspects of a woman's life and those associated with her (Kenney, 2000; Vuori et al., 2014; Greendale et al, 2010). Environment may place an additional burden affecting the health and balance of postmenopausal women (Mullings, McCaw-Binns, Archer, & Wilks, 2013; Vuori et al., 2014). Chronic disease causes issues with sleep patterns and increased stress (Przekop et al., 2012).

**Biomarkers.** Alpha-amylase (α-amylase) and cortisol are proteins in saliva identified as biomarkers for activity in the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis (Rohleder & Nater, 2009; Van Lenten & Doane, 2016). The secretion of these biomarkers generally follows a circadian pattern; levels are highest in the early hours of the day, decline slowly, and the lowest levels are in the evening hours (Kumari et al., 2009). Variance in sleep patterns and life stressors affect this pattern (Kumari et al., 2009). Salivary alpha-amylase (SAA) is consistently affected by true sleep disturbance according to one study (Seugnet, Boers, Gottschalk, Duntley, & Shaw, 2006). Additionally, Seugnet et al. (2006) reported that SAA levels were higher after sleep disturbance, but salivary cortisol (SC) levels did not change, indicating that it was the sleep disturbance, not stress, which caused changes in salivary α-amylase levels. Stress affects SC levels, but SAA levels are more directly associated with sleep (Lipschitz et al, 2013).

**Physical Activity.** Increased PA has a positive effect on the well-being of adults as demonstrated by improved sleep quality and decreased perceived stress (Sun, Norman, & While, 2013; Gregg, Pereira, & Caspersen, 2000; Rowe & Kahn, 1998). According to Ward-Ritacco, Adrian, Johnson, Rogers, & Evans (2014), improved physical functioning is associated with PA in middle-aged women. PM women can benefit from yoga exercises to manage stress and improve health (Rutledge, LaGuardia, & Bluestein, 2013; Mishra, Mishra, & Devanshi, 2011). PM women benefit from 150 minutes of PA weekly and should focus on endurance, strength, and balance exercises (Mishra, Mishra, & Devanshi, 2011). The Centers for Disease Control (CDC) and the World Health Organization (WHO) agreed with this amount of exercise for adults weekly to optimize health (CDC, 2015; WHO, 2015). Yoga has been identified as a leading PA to improve health and well-being among adults (Jeter, Nkodo, Moonaz, & Dagnelie, 2014; Sliwinski, Johnson, & Elkins, 2014; Ospina et al., 2008; Saper, Eisenberg, Davis, Culpepper, & Phillips, 2004; Birdee et al., 2008).

**Method**

This was a randomized, controlled trial with a yoga intervention for the treatment group and an educational offering for the attention control group. The yoga classes were weekly at a yoga studio for the intervention group; the educational course was offered weekly for the control group. Seven dependent variables included sleep duration, sleep quality, sleep depth, sleep restoration, stress, anxiety, and depression. Control variables were age, household type (living alone or with a partner), children, aging parents, health status (medical diagnosis), retirement status, race, ethnicity, and use of hormone replacement therapy (HRT).

**Sample**

The level of significance was set at 0.05 with 80% power to detect a moderate effect, thus the target sample was set at 60, with 30 in each group. Participants were recruited via radio announcements, newspaper ads, and flyers placed in churches, hospitals, and places of business. Two informational meetings were held to provide contact information to interested persons. Due to the reported low response rate on surveys (Holbrook, Krosnick, & Pfent, 2008), 25%

oversampling was planned to ensure a smaller margin of Type I or Type II error (Shi, 2008; Brink & Wood, 1998). Once enrolled, participants were randomly assigned to one of the two study groups.

**Intervention group.** Members of the intervention group attended a minimum of one 60-minute relaxation yoga class weekly for eight weeks. A certified yoga instructor consistently taught the classes at a professional yoga studio. The researcher directly observed attendance. Additionally, these participants practiced a minimum of three hours of yoga per week for eight weeks using an instructional DVD. Each participant in the intervention group received a packet of the health information taught to the control group, as well as a journal to document hours of sleep and personal reflections. The researcher held a weekly drawing for $10 gift card as an incentive for participants' time.

**Attention control group.** Members of the control group attended women's health classes for one hour per week. Additionally, they received a health manual with activities to address weekly for eight weeks. Examples included diet, exercise, health check-ups, etc. These women were also asked to journal over the course of the study. A drawing for a $10 gift card, as an incentive for their time, occurred weekly.

**Instrumentation.** Sleep was measured by self-reported hours of sleep, SAA levels, and the PROMIS-sleep disturbance tool at baseline and after the eight-week class. Stress was measured by SC levels, the PROMIS-anxiety tool, and the PROMIS-depression tool at baseline and after the eight-week class. Cronbach's alpha levels ranged from 0.85 to 0.95 on the PROMIS instruments in previous studies (PROMIS, 2019). The researcher observed participation and recorded yogic exercise in minutes each week for those assigned to the intervention group.

**Biomarkers.** Participants were requested to avoid caffeine, cigarettes, and alcohol for at least one hour prior to collecting saliva samples during the study because these could affect the results of the saliva samples (Salimetrics, 2016). Saliva samples were collected with the drool method and stored in the freezer and transferred on frozen cold packs to the Basic Science laboratory stored in the freezer at -80 degrees Celsius. The enzyme activity of α-amylase, the most abundant digestive enzyme in saliva, was quantified using a SAA kinetic enzyme assay kit purchased from Salimetrics. The intra-assay and inter-assay precision is reported as a coefficient of variation ranging from 2.5-7.2% (Salimetrics, 2016). This kinetic assay test involves taking readings at two different time points. It was critical that the researcher accurately recorded the time of the addition of the reagent and plate reading.

An enzyme-linked immunosorbent assay (ELISA) method using a kit from Salimetrics tested SC. Cortisol, the major corticosteroid produced in the adrenal cortex, has a circadian rhythm with highest levels in the morning and lower levels in the evening. However, levels vary independently of the circadian rhythm in response to stress. The intra-assay and inter-assay precision were reported as a coefficient of variation ranging from 3-11.

**Procedure**

The protection of participant rights was rigorously maintained. Informed consent was obtained from each participant. All data were stored securely in a locked file cabinet in a locked office in a limited access building and on a password-protected computer. Each participant was assigned a unique identifier that was used for data collection forms, data entry, and data storage.

The primary investigator randomized the biomarker samples both pre and post-intervention. At baseline, a research assistant randomly selected samples for each plate to be analyzed in the lab. Post-intervention, the research assistant carefully assigned a numeric code to each unique identifier. The primary investigator did not see the list of numeric codes and identifiers until all lab testing was completed.

Study flyers were placed in seven local churches, eight medical practices, and two local hospitals. Also, the primary investigator was interviewed on a local radio station to publicize the study. A total of 331 women were interviewed for this study; and 41 participants provided informed consent.

Then, each participant completed the following measures: average hours of sleep/night over the last week, the PROMIS-sleep disturbance scale, PROMIS-anxiety scale, and PROMIS-depression scale. Each instrument contained eight items each on a Likert scale of 0 to 5. The demographic form was the front of one page; thus, the paperwork took 20 to 35 minutes.

Participants were instructed in proper procedure for collecting saliva using the drool method. Saliva collection containers and storage bags were distributed to participants. Each participant received two red vials for day one, two yellow vials for day two, and two green vials for day three; each vial was labeled with the participant unique identifier and the day/time the sample was due. Saliva samples were collected twice daily to measure SAA and SC levels at baseline. Participants were instructed to collect saliva on Friday, Saturday, and Sunday upon awakening and at bedtime. The literature did not recommend any specific days of the week, therefore, the researcher chose these three days to capture the end of one traditional work week and the beginning of the next. Immediately after saliva collection, the sample was stored in the freezer. On the fourth day, the researcher retrieved all saliva samples, transferred them on ice to the laboratory, and stored them in a freezer at -80 degrees Celsius.

At the end of the last class, all participants reported average hours of sleep over the last week and completed the PROMIS-sleep disturbance, PROMIS-anxiety, and PROMIS-depression tools again. At the conclusion of the study, the researcher presented a gift certificate to one random winner for a spa day. All participants had an equal chance to win. A member of the intervention group won and mailed a hand written thank-you note to the researcher.

## Results

Categorical variables included race, ethnicity, household type, children living in the home, caring for aging parents, retired status, shiftwork, and hormone replacement therapy. The raw scores obtained from the PROMIS tools were converted to T-scores as directed by the PROMIS System. ANCOVA models were run to examine the interaction effect of categorical variables on sleep, stress, anxiety, and depression. Statistical Analysis System (SAS) 9.4 software was used to examine the study data.

**Demographics.** The groups did not differ at baseline. The mean age for women in the intervention group was 58.67 years and 60.07 years for women in the control group. Participants were largely Caucasian with 89% in the intervention group and 84% in the control group. Ethnicity majority was non-Hispanic with 94.7% in the intervention group and 92.3% in the control group.

Two participants in the intervention group withdrew due to physical illness – unrelated to study participation. Four women in the control group withdrew, citing family obligations, lost interest, and failure to respond to communication outreach. Three women in the control group did not turn in the second set of saliva samples. Therefore, 31 women completed the study. Table 1 shows equality of variance between intervention group and attention control group, except for the AM and PM cortisol samples in the pre-yoga group, which were lower than the control group.

Table 1. Equality of variances between the two groups.

| **Variable** | ***F* Value** | ***p* value** |
|---|---:|---:|
| Age | 2.11 | 0.1554 |
| Amylase Pre-Yoga | 1.85 | 0.2842 |
| **AM Cortisol Pre-Yoga** | **3.18** | **0.0471*** |
| **PM Cortisol Pre-Yoga** | **456.44** | **< 0.0001*** |
| Hours of sleep Pre-Yoga | 1.65 | 0.3345 |
| PROMIS Sleep Pre-Yoga | 1.80 | 0.2598 |
| PROMIS Depression Pre-Yoga | 1.22 | 0.7425 |
| PROMIS Anxiety Pre-Yoga | 1.31 | 0.5918 |

*$p < 0.05$

**Yogic Exercise, Sleep, and Stress**

The strength of the relationship between variables was examined with Pearson Correlation Coefficient (Table 2). In the intervention group, a strong inverse correlation existed in hours of sleep from pre to post-intervention . A moderate inverse correlation was noted in the change of PROMIS-depression scores. The number of hours spent in yogic exercise were correlated with change in PROMIS sleep score ($p < 0.0001$), and change in PROMIS depression score ($p = 0.0303$).

Table 2. Association of Pre-Yoga and Post-Yoga scores using Pearson Correlation Coefficient.

| **Variable** | ***r*** | ***p* value** |
|---|---:|---:|
| Change in PROMIS Sleep score | -0.2647 | 0.1575 |
| Change in PROMIS Anxiety score | -0.2690 | 0.1506 |

| **Variable** | ***r*** | ***p* value** |
|---|---|---|
| **Change in PROMIS Depression score** | **-0.3960** | **0.0303*** |
| **Change in Hours of Sleep** | **-0.7717** | **< 0.0001*** |

* $p < 0.05$

In the yoga group, statistically significant effects were noted between hours of sleep and retired status ($p = 0.0356$), hours of sleep and household status ($p = 0.0328$), and hours of sleep with group type ($p = 0.0266$). The yoga group had improved sleep compared to the attention-control group. However, Bonferroni post hoc t-tests for change in hours of sleep did not reveal significance. The Bonferroni correction was used to adjust probability values because of the increased risk of a type I error when making multiple statistical tests (Shi, 2008; Trochim & Donnelly, 2008).

Reduced general linear modeling showed that the change in PROMIS sleep score was affected by living with a child ($p = 0.0393$), medical diagnosis ($p = 0.0333$), and race ($p = 0.0244$). Bonferroni post hoc tests indicated a statistically significant difference in change in PROMIS depression scores and race. The difference between means in Black participants was 11.450, with a 95% confidence interval of 0.274 and 22.626.

Medical diagnosis ($p = 0.0487$) and caring for a parent ($p = 0.0397$) were indicated as significantly associated with the PROMIS anxiety scores. Change in alpha amylase (Table 3) demonstrated significant results in race ($p = 0.0174$) and alpha-amylase pre-yoga ($p < 0.0001$).

Table 3. Reduced general linear model with dependent variable: Change in SAA.

| **Source** | DF | Mean Square | ***F* value** | ***p* value** |
|---|---|---|---|---|
| Caring for a parent | 1 | 5053.14571 | 1.70 | 0.2036 |
| **Race** | 2 | 14118.10929 | **4.75** | **0.0174*** |
| **Alpha-amylase pre-yoga** | 1 | 64071.43931 | **21.57** | **< 0.0001*** |

* $p < 0.05$

While exploring effect of the yoga intervention with Change in PM Cortisol as the dependent variable (Table 4), PM Cortisol pre-yoga intervention was statistically significant (p = < 0.0001). The change in diurnal cortisol (Table 5) was not significant.

Table 4. Reduced general linear model with dependent variable: Change in PM Cortisol.

| **Source** | DF | Mean Square | ***F* value** | ***p* value** |
|---|---|---|---|---|
| Living with child | 1 | 0.00923380 | 1.61 | 0.2183 |
| Household situation | 2 | 0.00838494 | 1.46 | 0.2543 |
| Medical diagnosis | 1 | 0.00312747 | 0.55 | 0.4683 |
| Caring for a parent | 1 | 0.00880637 | 1.54 | 0.2289 |
| Retired | 1 | 0.02076923 | 3.62 | 0.0708 |
| Shift work | 1 | 0.00488904 | 0.85 | 0.3663 |
| **PM Cortisol pre-yoga** | 1 | 18.18616 | **3172.19** | **< 0.0001*** |

* $p < 0.05$

Table 5. Change in diurnal cortisol range and ratio.

| **Variable** | Method | Variances | DF | ***t* value** | ***p* value** |
|---|---|---|---|---|---|
| Change in cortisol range | Pooled | Equal | 29 | -0.80 | 0.4300 |
| Change in cortisol ratio | Pooled | Equal | 29 | -0.13 | 0.8937 |

## Discussion

The purpose of this study was to examine the effect of a yoga intervention on sleep, stress, anxiety, and depression in post-menopausal (PM) women aged 40 years and older. Aims of the study were: (1) Determine the effect of yoga on sleep, as measured by salivary alpha-amylase levels, PROMIS-Sleep Disturbance scores, and self-reported hours of sleep; and (2) Determine the effect of yoga on stress levels, as measured by salivary cortisol levels, PROMIS-Anxiety scores, and PROMIS-Depression scores. We met these aims with noted limitations. The four hypotheses that guided this study and results are as follows:

1. PM women participating in an 8-week yoga class will have better sleep compared to an attention control group. This hypothesis was supported in sleep duration but not on PROMIS tool for sleep disturbance.
2. PM women participating in an 8-week yoga class will have lower levels of stress compared to an attention control group. Not supported.
3. PM women participating in an 8-week yoga class will have lower levels of anxiety compared to an attention control group. Not supported.
4. PM women participating in an 8-week yoga class will have lower levels of depression compared to an attention control group. Not supported.

The intervention and control groups were similar at baseline. A stem-leaf plot demonstrated approximate normal distribution, so parametric tests were used for this data. Pearson Correlation Coefficients revealed the yogic intervention correlated with the change in total hours of sleep ($p < 0.0001$) and in PROMIS depression scores ($p = 0.0303$). Initially, statistically significant effects were noted between hours of sleep and retired status ($p = 0.0356$), household status ($p = 0.0328$), and group type ($p = 0.0266$). The yoga group had improved sleep compared with the control group. However, Bonferroni post hoc t-tests for change in hours of sleep did not reach significance.

Salivary alpha-amylase is released into the saliva by stimulation of the sympathetic nervous system and was examined as an indicator of sleep disturbance. Change in alpha amylase demonstrated statistically significant results by race ($p = 0.0174$) and alpha-amylase pre-yoga ($p < 0.0001$), but Bonferroni t-tests did not detect any difference. SAA results were positively affected by yogic exercise according to race for some participants. However, the limited sample prohibits generalizability.

Salivary cortisol (SC), a product of stimulation of the limbic-hypothalamic-pituitary axis, was examined as an indicator of stress in the study participants. The results examining change in cortisol did not detect any differences in salivary samples collected pre-yoga compared to salivary samples collected post-yogic intervention. Although the participants documented in journals a palpable decrease in stress, the SC levels did not reflect it.

The actual number of hours of sleep increased in the control group. Participants shared with the researcher that the relaxation techniques of yogic breathing and meditation practice aided them in going to sleep initially, as well as going back to sleep when awakened during the night. Additionally, women reported that the social aspect of the yoga classes was supportive over time. They developed relationships with each other, telephoning to schedule extra yoga practice together. The socialization of these PM women provided an unexpected benefit for sleep in the study. The participants found commonalities and shared ideas to cope with aging parents, work stress, and children.

Participants were instructed to collect specific amounts of saliva upon awakening and before bed on Friday, Saturday, and Sunday before and after the intervention. Collection tubes were color-coded: red for day one, yellow for day two, and green for day three. Each tube was labeled with the participant's code and identified as AM sample or PM sample. Participants shared the color coding and sample identification (AM and PM) was very helpful.

Although careful instructions were given for saliva collection, several participants in the control group did not contribute the correct amount of saliva, making the sample unusable. Others did not contribute three days of saliva samples. Originally, the researcher intended to run SC and SAA tests in triplicate but was able to run duplicates only due to missing samples. After the study was over, three of these participants shared they did not think their samples would be important since "we were just in the control group." However, other members of the control group shared how beneficial the education classes were, especially the classes on meditation/relaxation, diet/exercise, and wellness checks. For future studies, it will be important that participants be reassured throughout the study and demonstrate understanding of the value of their consistent participation and adherence to instructions.

## Limitations

**Sample**. Sample size was a limitation for this study. The rurality of the region made sample collection challenging. Additionally, several people withdrew from the project during the 8-week intervention period. Most of these withdrawals were from the attention control group, even though the education class was interactive and engaging. The researcher discovered during week 6 of the study that several members of the group wanted to "quit and take up yoga." Participants in the attention-control group were encouraged to take free yoga lessons from the same instructor once the study was completed.

A search for participants was conducted over six weeks. A total of 331 attended the informational meetings for the study. Sixty participants were recommended for the sample size of this study; 41 women agreed to participate in the study and signed informed consent. However, only 38 participants provided saliva samples prior to the intervention. Since these 38 were prepared for the study, the decision was made to conduct the study with what was available. All options to acquire participants had been exhausted in both rural parishes identified as the study community.

It is important to note the effect the human factor had on this study. The PM women were multi-taskers; this study added another layer of tasks they needed to accomplish regularly for eight weeks. There may be factors unknown to the researcher that interfered with an individual's motivation to perform the yogic exercise or collect saliva samples as instructed. Environmental factors may have affected the intervention, exacerbated by individual role responsibilities.

**Self-Report Instruments.** The PROMIS-sleep disturbance, PROMIS-anxiety, PROMIS-depression, and demographic surveys may be a threat to validity/reliability because the response rate and type of respondent may indicate a selection bias; plus, these were all dependent on self-report. Participants may have answered the tools based on what they think they should answer (Shi, 2008; Trochim & Donnelly, 2008).

**Laboratory Tests.** The researcher was not able to perform kinetic assay tests and ELISA tests in triplicate as planned due to missing saliva samples. Therefore, the tests were run in duplicate. This is concerning, because there was a wide variation among individual sample results. This may have been better explored with appropriate amount and number of samples consistently among the participants.

## Applied Recommendations

Women may be able to enhance qualities of sleep while decreasing stress through yogic physical activity. Recommendations can direct women to incorporate regular yogic exercise in their daily routine. Education may include directing women to government initiatives for advice and direction. Health care professionals can engage women in dialogue to encourage realistic goals for physical activity while considering physical ability or cultural expectations. Women and the healthcare team may consider discussing potential barriers and ways to monitor activity to aid the women to stay on track towards their goals.

## Recommendations for Future Research

Future studies should consider specific methods to address proper saliva collection with adherence to protocol. Color coding and pre-labeling the collection tubes worked very nicely and is recommended for future work. Does it matter what day of the week saliva samples are collected? Shift workers might have the same stress on a Saturday that a businesswoman has on Monday. Does working the night shift affect SC and SAA in a participant? Future studies might consider all participants working the same schedule for uniformity. Future work should run ELISA tests and kinetic assay tests in triplicate.

Sample selection should achieve the numbers needed to determine significance. Perhaps the researcher could stage the study in four-week sessions: starting a new group every four weeks until the proper sample size has been reached. More attention to sustaining engagement, especially in the attention control group, is warranted. Perhaps, a focus group with similar women to explore options for this threat would be beneficial. It is critical that every participant understand the value they have in the study and the importance of their contribution.

Participants were excited to engage in yogic exercise but wanted to see more immediate results. Therefore, future studies might consider walking, water exercise, or ballroom dancing, as options. Participants might see visible physical results, as well as enhancing quality of life characteristics such as improved sleep. This might serve to encourage them to adhere to study protocol.

## Conclusion

Questions remain concerning the effect of yogic exercise on sleep and stress as measured in SAA and SC. Continued research is needed in areas such as sleep quality, sleep depth, and sleep duration. Studies are also needed to investigate clinical outcomes of physical activity measuring SAA and SC over the course of three to six months. The adverse effects of sleep disturbance and stress have been widely recorded. However, continued research is needed to prevent the consequences of daily life stressors for PM women.

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