Assessment of Effects of Carbon Dioxide Exposure on Sleep Stability in Insomnia Using the Envelope Analysis

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Objectives: Insomnia is a common sleep disorder, and various strategies have been explored for its treatment. One of the methods involves the administration of carbon dioxide (CO2) at bedtime, as the CO2 increment exerts a sedative effect on the central nervous system. We aimed to assess the effect of CO2 on sleep quality in patients with insomnia using the coefficient of variation of the envelope (CVE) analysis, a novel analytical method. Methods: In this randomized, double-blind, sham-controlled crossover trial, 24 adults with sleeping difficulties underwent level 1 polysomnography. Two interventions involved exposure to either 2% low-concentration CO2 gas or room air. CVE analysis was performed to assess the stability of the delta band. Morphological variations in delta waves can be quantitatively monitored using the envelope analysis, which assesses the effect of manipulations on sleep that may otherwise elude detection. Results: Exposure to CO2 improved the total sleep time (TST) (p=0.010) and total arousal index (TAI) (p=0.011). The CVE analysis showed a distinct distribution between the experimental and sham-controlled groups. Moreover, correlations between the TST and CVE (r=0.41) and those between the TAI and CVE (r=0.40) were observed. Conclusions: This study is the first to evaluate the effects of CO2 exposure on sleep stability in patients with insomnia. The CVE analysis implies the possibility of sleep stabilization by CO2 administration and suggests a link between enhanced sleep maintenance and sleep stability.

Keywords: Insomnia; Carbon dioxide; Sleep stability; Envelope analysis.

INTRODUCTION

Sleeping difficulties are prevalent in the general population worldwide.1 About one-third of the adult population is estimated to experience at least one of the insomnia symptoms (difficulty in initiating or maintaining sleep, associated daytime fatigue, and dysfunction).2 The prevalence of insomnia has increased over the last few decades,3 with the coronavirus disease 2019 pandemic exacerbating the issue.4 As public awareness and concern regarding the importance of quality sleep have increased, various insomnia management strategies have been developed. One such strategy involves exposure to carbon dioxide (CO2) during bedtime. CO2 increment exerts a sedative effect on the central nervous system. At the initiation of sleep, the breathing rate and ventilation decrease, leading to an increase in alveolar partial pressure of CO2 (pCO2). The sedative effect becomes more pronounced with increased CO2 concentration in the air.5 A previous study conducted in patients with insomnia reported shortened sleep-onset latency in hypercapnic breathing-induced groups.6 Additionally, exposure to CO2 for 10 min during the daytime results in changes in electroencephalography (EEG) tracing and an increase in delta rhythm.6 However, methods for objectively confirming the effects of CO2 exposure at bedtime in patients with insomnia, in terms of sleep initiation and maintenance, have not been established.

A previous animal study demonstrated that the administration of 2% CO2 increased the total sleep time (TST) and decreased awakening7 by stimulating the release of adenosine,8 a potent endogenous somnogen. Moderate hypercapnia also increases rapid eye movement (REM) sleep periods in cats by hyperpolarizing REM-off neurons and initiating REM sleep.9 These explanations are based on microscale hypotheses. Hence,
further studies on the mechanism underlying the effect of CO₂ exposure on human sleep at the macroscale are needed.

Polysomnography (PSG) is the conventional gold standard test for objectively measuring of sleep disorders. However, the conventional PSG scoring systems have inherent limitations, as they rely on visual inspection and yield semiquantitative parameters that may hinder the detection of subtle differences. Hence, novel sleep biomarkers have been introduced for more accurate analysis. A novel computational analysis method for EEG, the coefficient of variation of the envelope (CVE), provides a scale-independent measure of temporal stability and has recently been applied in sleep analyses with promising results. In a previous study comparing the sleep architecture of younger and older adults, significant differences were observed in CVE values between age groups, with decreases in slow-wave sleep (SWS) stability and delta wave amplitude in older adults. Another study revealed the effect of exercise on sleep using CVE analysis and demonstrated increased SWS stability with lower CVE values in the exercise group compared with the control group.

We aimed to measure the effect of CO₂ exposure at bedtime in patients with insomnia through an objective test and subsequent analysis using the CVE.

**METHODS**

**Study design and participants**

A double-blind, randomized, sham-controlled crossover study was conducted. Written informed consent was obtained from all participants, and the study was approved by the Institutional Ethics Committee of Samsung Medical Center (2021-04-133). The participants were recruited through public notices. Individuals aged 35–65 years, who volunteered to participate in the study, understood the study purpose, and reported subjective insomnia symptoms with a Pittsburgh Sleep Quality Index (PSQI) score of 5 were enrolled in the study. Conversely, individuals who met any of the following criteria were excluded from the study: 1) engaged in shift work, 2) pregnant or in the postpartum period, 3) diagnosed with pulmonary disease, 4) developed unstable or severe medical illness (cardiac, nephrology, gastrointestinal disease, diabetes mellitus, hypertension, thyroid disease, immunological disease, and cancer), 5) experienced cognitive decline and progressive psychological or neurological disease, 6) diagnosed with severe snoring, REM sleep behavior disorder, narcolepsy, and circadian rhythm sleep disorder, and 7) had taken sleeping pills within 4 weeks.

Both the participants and sleep specialists who interpreted the PSG results were blinded. Each individual underwent two PSG sessions, with a 2-week interval between the sessions. The order ratio was 1:1 using block randomization with the R program (version 3.3.2, R Core Team, R Foundation for Statistical Computing, Vienna, Austria; www.r-project.org). The participants completed a sleep diary 1 week before the first PSG night and during the 2-week interval until the second PSG night. Alcohol consumption was prohibited during the study period.

**CO₂ exposure**

The device for gas exposure was sleep air (GoSleep®, NYX, Hanam, Korea). Sleep air atomizes CO₂ gas and is composed of a main body that contains a gas cylinder (18.6×24.0×69.5 cm) and a leg (29.4 cm). The PSG room was ventilated by a central air control system, with individual air conditioners installed in each room. Throughout the study, the room temperature was kept between 24.5°C and 25.5°C, while the relative humidity was maintained at 45%. The room was soundproofed, and the sound level ranged 35–55 dB during the study. The device was activated when the lights were turned off. In the first phase, room air was sprayed with a gradual increment in wind strength for 3 min. In the second phase, a 2% CO₂ mixture gas was sprayed for 15 min on the experimental group, while room air without a mixture of CO₂ was sprayed on the sham-controlled group. The CO₂ spray duration was based on the United States National Institute for Occupational Safety and Health, which established a short-term exposure limit of a maximum of 30,000 ppm of CO₂ within 15 min. Additionally, results of a previous meta-analysis on the mean sleep onset latency of healthy adults aged 35–65 years (35–49 years: 14.4 min and 50–64 years: 15.7 min) were considered. In the final phase, room air was sprayed with a gradual decrease in wind strength for 5 min. Indoor CO₂ concentration changes were monitored for 1 min using a certified gas analyzer (certificate no. SC2112-29906-1; NYX, Hanam, Korea).

**Parameters**

**PSG**

PSG was conducted using Embla N7000 (Medcare-Emb- la®, Reykjavik, Iceland) with six-channel EEG, two-channel electrooculography, and a minimum of four-channel electromyography for the chin, intercostal muscles, and both anterior tibialis. The PSG data were manually scored according to the American Academy of Sleep Medicine guidelines. The following PSG data were collected: TST, arousal index (AI) (total, respiratory, spontaneous, and movement arousals), sleep efficiency, sleep latency (SL), wakefulness after sleep onset, sleep stage (N1, N2, N3, and REM sleep [%]), apnea-hypopnea index, and periodic limb movement index.
**Sleep questionnaire**

Before the PSG, the validated Korean versions of the Epworth Sleepiness Scale (ESS),\textsuperscript{20} Insomnia Severity Index (ISI),\textsuperscript{21} and PSQI,\textsuperscript{22} and the Korean version of the Beck Depression Inventory\textsuperscript{23} were conducted. Before and after PSG, subjective sleepiness and alertness were assessed using the Karolinska Sleepiness Scale (KSS).\textsuperscript{24}

**CVE**

The CVE of the delta EEG band was calculated based on the C3-A2 EEG recordings at 30-s intervals. To mitigate the aliasing effects, the epochs had a 50% overlap, meaning that the epoch length was 60 s. Initially, each epoch was digitally bandpass filtered (delta band: 0.5–4.0 Hz) with a fourth-order infinite impulse response implementation of a Butterworth filter using the “signal” package in the R language. Subsequently, the envelope of the filtered EEG was derived using the Hilbert transform according to the standard relation. The mean and standard deviation (SD) of the obtained envelope were calculated, and a normalized version of the CVE was obtained using the following equation, with 0.523 representing the value for Gaussian waves according to the standard relationship:

\[
\text{CVE} = \frac{\text{SD}}{\text{mean}} \times 0.523
\]

Consequently, CVE values > 1 indicate processes more phasic than Gaussian waves, while values < 1 suggest more sinusoidal processes. For each epoch, the coefficient of variation (SD/mean) of the corresponding envelope is stored as the relevant feature.\textsuperscript{14}

**Statistical analysis**

Statistical analyses were performed using SPSS (version 20.0; IBM Corp., Armonk, NY, USA). Continuous variables were expressed as numbers (%) or the mean±SD. A paired t- or Wilcoxon signed-rank test was used for comparison between the two groups. Correlation analysis was performed between the CVE and significant sleep parameters in the PSG. A \(p\) value of <0.05 was considered significant.

**RESULTS**

**Participants and sleep questionnaires**

This study included 24 participants, with a mean age of 55.4±6.3 years and with women comprising 62.5% of the sample. The obtained ISI score was 14.9±4.7, which showed moderate severity of insomnia. The PSQI scores were 10.0±3.3 and ESS 11.3±5.4, representing overall sleep quality impairment and daytime sleepiness. The participants showed mild depressive mood with a BDI score of 14.8±8.6. None of the participants were excluded based on the BDI score, as none were diagnosed with depressive disorder. Moreover, mild depres-

<table>
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<th>Sham-controlled</th>
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<tr>
<td>Time in bed (min)</td>
<td>361.3±55.2</td>
<td>347.2±35.7</td>
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<tr>
<td>TST (min)</td>
<td>311.0±74.3</td>
<td>287.2±69.4</td>
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<td>Sleep onset latency (min)</td>
<td>9.0±12.9</td>
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<td>WASO (%)</td>
<td>12.9±9.9</td>
<td>16.3±16.3</td>
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<tr>
<td>Sleep efficiency (%)</td>
<td>84.9±11.6</td>
<td>82.0±16.9</td>
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<td>Sleep stage (%)</td>
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<tr>
<td>N1</td>
<td>16.2±9.0</td>
<td>20.8±17.8</td>
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<td>N2</td>
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<td>N3</td>
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<td>REM</td>
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<td>TAI (/h)</td>
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<td>12.3±12.8</td>
<td>13.9±14.8</td>
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</table>

*\(p\) <0.05. PSG, polysomnography; TST, total sleep time; WASO, wakefulness after sleep onset; REM, rapid eye movement; TAI, total arousal index; AI, arousal index; NREM, nonrapid eye movement
sive mood did not affect the sleep parameters compared with severe depression in insomnia.\textsuperscript{25} The KSS scores before and after PSG did not differ between the two groups (KSS before PSG: 4.6±1.6 [experimental group] vs. 4.9±2.0 [sham-controlled group], KSS after PSG: 4.8±1.5 [experimental group] vs. 5.0±2.2 [sham-controlled group], \( p > 0.05 \)).

Conventional PSG parameters
Exposure to low concentrations of CO\(_2\) improved the TST (311.0±74.3 vs. 287.2±69.4, \( p = 0.010 \)) and total arousal index (TAI) (19.7±9.5/h vs. 24.0±13.7/h, \( p = 0.011 \)). However, no other parameters, including SL, significantly differed between the two groups (Table 1).

Sleep stage comparison of each sleep cycle
The duration of each sleep stage (wake, N1, N2, N3, and REM) of consecutive sleep cycles is shown in Fig. 1. In the first sleep cycle, no significant differences were observed between the two groups (Fig. 1A). In the second cycle, the wake time was significantly shorter in the experimental group compared with that in the sham control group (\( p < 0.05 \)) (Fig. 1B). Although the difference was not significant, N1 was shorter in the experimental group. In the third cycle, REM sleep was longer in the experimental group (\( p < 0.05 \)) (Fig. 1C).

Delta amplitude and CVE density heatmaps
The density of plots, represented as a heatmap-like coloring of the markers displayed between the delta band amplitude and CVE, showed a distinct distribution between the experimental and sham-controlled groups. An upper-left cluster (lower CVE<0.6 and higher delta amplitude>2.3) was observed in the experimental group (Fig. 2). However, no significant dif-

![Figure 1. Comparison of composition of each sleep cycles between experimental versus sham controlled group. A: 1st sleep cycle. B: 2nd sleep cycle. C: 3rd sleep cycle. *\( p < 0.05 \). REM, rapid eye movement.](https://www.e-jsm.org)
Difference were observed in the average CVE during sleep. Correlations were observed between the TST and CVE ($r=0.41$) (Fig. 3A) and between the AI and CVE ($r=0.40$) (Fig. 3B).

**DISCUSSION**

The present study investigated the effect of low-dose CO$_2$ (2%) exposure before bedtime on sleep initiation and maintenance in patients with insomnia. The magnitude of this effect was assessed using conventional PSG parameters, sleep stage composition, and novel EEG envelope analysis.

The conventional PSG parameters implied sleep maintenance improvement following exposure to 2% CO$_2$ before bedtime, with the experimental group showing a lower AI and longer TST compared with those in the sham-controlled group. At the initiation of sleep, ventilation decreased, and alveolar pCO$_2$ levels increased. Thus, CO$_2$ inhalation at bedtime was mainly assumed to affect sleep initiation and targeted sleep induction. In insomnia patients, the hypercapnic breathing-induced groups showed shorter sleep onset latency compared with the normal breathing group; however, the result was based on subjective reports rather than objective markers. Another study reported that CO$_2$ inhalation at the initiation of sleep increased the slow waves on EEG, leading to sleep induction in healthy adults. Unlike previous findings, our study showed no differences in objective or subjective SL improvement in the CO$_2$-exposed group, warranting further investigation.

The average SL of the participants was $9.0\pm 12.9$ min, which was shorter than the duration of CO$_2$ exposure time in the second stage of the experiment (18 min). Consequently, CO$_2$ inhalation continued after the onset of sleep in these participants. Notably, the nonrapid eye movement (NREM) AI significantly decreased in the experimental group.
sleep is more prevalent in the earlier phase than in the later phase of sleep, these results imply the possibility that CO$_2$ exerts a sleep-maintaining effect in the earlier phase of sleep beyond sleep initiation. The comparison of the sleep stages of each sleep cycle between the two groups provided a more obvious view of the effect of low-dose CO$_2$ on sleep maintenance. In this study, the number of awakenings significantly decreased during the second cycle. These results provide insights into the timing of the CO$_2$ effect on sleep, which, to the best of our knowledge, has not been suggested in previous studies. The sleep-maintaining effect occurs in the second cycle of sleep by reducing awakening, which is represented as a NREM AI reduction in the PSG parameters. A previous study, with results that corroborate with our findings, reported that the administration of 2% CO$_2$ increased the TST by 21.0% and decreased the awakening duration by 16.2% in cats.

This result is attributed to the release of adenosine, which endogenous somnogen that affects sleep by acting on the basal forebrain and adenosine-sensitive areas, stimulated by a mild hypercapnic state. The duration of REM sleep was increased in the CO$_2$-exposed group during the third sleep cycle. Because REM sleep is more prevalent in the later phase of sleep, a significant difference in the amount of REM sleep between the two groups would have been observed in the later cycle. In addition, the duration of REM sleep correlates with CO$_2$ decrement in cats, and moderate hypercapnia increases the duration of REM sleep. This is explained by the fact that increased CO$_2$ levels during the NREM sleep period may hyperpolarize REM-off neurons and initiate REM sleep. These findings support the results of the present study. However, previous explanations are based on a microscale hypothesis, necessitating a macroscale analysis of human sleep. Thus, we analyzed a novel EEG marker, as CVE analysis has the potential to provide a clearer answer and broader perspective.

Recently, a novel computational approach for assessing brainwave stability through envelope analysis was proposed. The CVE is a scale-independent measure of the temporal stability of oscillations. Lower CVE values are associated with stable sinusoidal oscillations, intermediate values indicate Gaussian oscillations, and high CVE values suggest irregular phasic processes. We previously reported a significant increase in SWS stability during the early phases of sleep following acute exercise based on envelope analysis. Another recent study reported a decrease in SWS stability in older adults compared with their younger counterparts, accompanied by a decrease in delta wave amplitude. These findings demonstrate that CVE can directly reflect sleep depth and stability. However, in the present study, our findings regarding the EEG delta band stability did not differ between the two groups, as evaluated through envelope analysis. This result may imply that CO$_2$ exposure might not solely affect delta waves during sleep, but could also influence faster waves and oscillations during sleep. Nevertheless, the TST and CVE showed moderate negative correlations ($r=0.41$) (Fig. 3A), whereas the AI and CVE values showed moderate positive correlations ($r=0.40$) (Fig. 3B). These findings a potential association between CVE in insomnia patients and wakefulness, which increases the CVE due to the abrupt large oscillations in the EEG during waking periods. Although the present study did not detect a significant discrepancy in the CVE between the two groups, a difference was found in the cluster of each plot (epoch), with higher amplitude ($>2.3$) and lower CVE ($<0.7$) in the CO$_2$-exposed groups. This finding suggests a tendency towards enhanced stability of SWS. Collectively, these findings indicate the promotion of sleep stabilization with increased SWS power.

A limitation of our study is the small sample size. Future studies with a larger sample size are likely to unveil more significant differences between the two groups. Another limitation was the composition of the participants. Among the insomnia symptoms, approximately half of the participants reported experiencing early awakenings and difficulty falling asleep, while a relatively smaller proportion of participants (approximately one-third) reported difficulties in initiating sleep. Thus, a more balanced distribution of insomnia symptoms may yield more accurate results. Finally, the intervention was administered only once. Considering the nature of sleep homeostasis, a one-night exposure to CO$_2$ may not be sufficient to result in significant changes in sleep structure. Further studies involving multiple nights of exposure are warranted.

To our knowledge, this study is the first to evaluate the effects of CO$_2$ exposure on sleep stability in patients with insomnia. CO$_2$ exposure is effective in improving sleep maintenance by extending the TST and reducing arousal. CVE analysis implies the possibility of sleep stabilization by CO$_2$ administration and suggests a link between enhanced sleep maintenance and sleep stability. Based on these findings, the development of safe and effective CO$_2$ devices that can be widely used in sleep clinics is required.

**Conflicts of Interest**

Eun Yoon Joo, the Editor-in-Chief of the *Journal of Sleep Medicine*, was not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

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**Author Contributions**

Envelope Analysis and Sleep Stability

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