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ORIGINAL RESEARCH

Cerebrospinal Fluid Nitric Oxide Synthase is a Potential Mediator Between Cigarette Smoke Exposure and Sleep Disorders

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Objective: Cigarette smoking and low peripheral nitric oxide synthase (NOS) levels are strongly associated with sleep disorders. However, whether cerebrospinal fluid (CSF) NOS relates to sleep disorders and whether CSF NOS mediates the relationship between cigarette smoking and sleep disorders is unclear.

Methods: We measured CSF levels of total NOS (tNOS) and its isoforms (inducible NOS [iNOS] and constitutive NOS [cNOS]) in 191 Chinese male subjects. We applied the Pittsburgh Sleep Quality Index (PSQI).

Results: The PSQI scores of active smokers were significantly higher than those of non-smokers, while CSF tNOS, iNOS, and cNOS were significantly lower (all $p < 0.001$). CSF tNOS, iNOS, and cNOS were negatively associated with PSQI scores in the general population (all $p < 0.001$). Mediation analysis suggested that CSF tNOS, iNOS, and cNOS mediate the relationship between smoking and PSQI scores, and the indirect effect accounted for 78.93%, 66.29%, and 81.65% of the total effect, respectively.

Conclusion: Cigarette smoking is associated with sleep disorders. Active smokers had significantly lower CSF levels of tNOS, iNOS, and cNOS. Furthermore, tNOS, iNOS, and cNOS mediate the relationship between cigarette smoking and sleep quality. This study provides insights into how cigarette smoke affects sleep disorders.

Keywords: cerebrospinal fluid nitric oxide synthase, cigarette smoking, Pittsburgh Sleep Quality Index, sleep disorders, mediation

Introduction

Cigarette smoking is the most preventable cause of disease and death worldwide.[1] Clinical and laboratory studies showed that cigarette smoking is significantly associated with the risk of psychiatric disorders, including sleep disorders.[2–4] Long-term smoking causes poor sleep quality, initiation, maintenance impairment, and daytime sleepiness.[5,6] However, little is known about the mechanisms between cigarette smoke and sleep disorders. There are currently two possible explanations: changes in the chemistry of the nervous system and incidence of respiratory problems.[7]

Cigarette smoke contains thousands of chemicals that produce several free radicals and reduce nitric oxide synthase (NOS) activity and expression.[8,9] There are two forms of NOS synthase: (i) constitutive NOS (cNOS) (including neuronal [nNOS] and endothelial [eNOS]) and (ii) inducible NOS (iNOS), which responds to the generation of nitric oxide (NO).[10,11] Animal and cell experiments showed that cigarette smoke exposure can decrease cNOS and iNOS.[8,12,13] Many laboratories have developed evidence linking NOS to sleep disorders. NOS is a calcium/calmodulin-dependent

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enzyme responsible for nitric oxide (NO) biosynthesis from L-arginine.[14] The literature suggests that NOS is associated with sleep disorders.[15,16] Studies showed that iNOS and nNOS, although synthesizing the same messenger NO, play distinct roles in sleep regulation.[11] The iNOS is produced by microglia and astrocytes, which ensures adequate rapid eye movement (REM) sleep and increases slow-wave sleep.[11] The nNOS is distributed throughout the brain, with the highest distribution in the pons, where it maintains daily homeostatic sleep (including slow-wave and REM sleep).[11] In human umbilical vein endothelial cells subjected to intermittent hypoxia, NOS activity levels were significantly lower, and eNOS mRNA expression was downregulated.[16] In clinical studies, patients with obstructive sleep apnea significantly decreased peripheral eNOS levels.[17]

Cigarette smoke enters the brain quickly by many pathways and may cause a fall in CSF total NOS, iNOS, and cNOS levels.[18] However, no study has yet used CSF NOS to investigate the relationship between active smoking and sleep disorders. Therefore, we determined the associations between cigarette smoking and sleep disorders by measuring CSF NOS.

Methods

Participants

Considering the low proportion of female smokers in China (3.2%), we enrolled only male subjects. We recruited 191 Chinese men (age range 17–64 years) scheduled for anterior cruciate ligament reconstruction surgery from September 2014 to January 2016. Of these, 87 were active smokers and 104 were non-smokers. Subjects who had never smoked were considered non-smokers. According to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders, subjects who consume half a pack of cigarettes (ie, ten cigarettes) or more per day for more than a year are active smokers. For the purposes of this study, people who have ever smoked (ie, who have a history of smoking but have stopped smoking) were excluded to accentuate the difference between active smokers and non-smokers. The exclusion criteria were as follows:1) a history of drug abuse or dependence; 2) a family history of neurological diseases or psychosis; 3) systemic or central nervous system diseases determined using the Mini-International Neuropsychiatric Interview.

We recorded age, body mass index (BMI), marriage, living status, and clinical data, including blood pressure, Pittsburgh Sleep Quality Index (PSQI) Global Score, and biochemical indicators. The Institutional Review Board of Inner Mongolian Medical University approved this study, and participants provided written informed consent before data collection.

Assessments, Biological Sample Collection, and Laboratory Tests

Active smokers provided the age at which they started smoking, the years of active smoking, the average number of cigarettes they smoked daily, and the maximum amount of tobacco per day. The PSQI measures sleep quality in the previous month. We used the Chinese version of the questionnaire and asked subjects to make choices on a four-point Likert scale (from 0 = "no difficulty" to 3 = "severe difficulty") one day before CSF extraction. Combining the resulting data generates seven subscales: subjective sleep quality, habitual efficiency, latency, use of sleep medication, duration, disturbances, and daytime dysfunction. The total score ranges from 0 to 21, with higher scores indicating poorer sleep quality. A PSQI score of 5 and above is considered a clinically relevant sleep disorders.[19,20] Sleep quality and latency fractions ≥2 were defined as low sleep quality and long sleep latency, respectively. Sleep efficiency < 85% was defined as low sleep efficiency. The onset of subjective sleep disturbance within the last month was described as a sleep disturbance. The use of sleep medication in the previous month was defined as the use of sleep medication. Daytime activity disorders in the previous month was described as a daytime activity disorders.

Before cruciate ligament reconstruction, a standard clinical procedure, CSF samples were obtained by lumbar puncture. The day before surgery, an anesthesiologist administered 3 mL 0.5% ropivacaine locally, collected 5 mL of CSF from the intrathecal space, and immediately froze the sample at –80 °C.

After extraction, routine, biochemical, and cytological examinations were performed on the CSF, and nitrate reductase was used to measure. The method was as follows: NOS catalyzes the reaction of L-arginine with molecular oxygen to

generate NO, which continues to create colored compounds. We configured the reagents according to the labeling method of the reagent kit and used a colorimetric method to detect the absorbance values of each tube. We calculated NOS content according to the formula given in the manual. The Nanjing Jiancheng Bioengineering Institute provided the NOS kit. Laboratory technicians were blinded to clinical data. These methods were described in detail previously.[18,21]

Statistical Analysis

We used the Mann–Whitney rank-sum and Chi-square tests to compare the two groups' general demographic data, clinical data, and raw biomarkers. Spearman correlation analysis calculated the relationship between raw biomarkers (total NOS [tNOS], iNOS, and cNOS) and PSQI scores. The tNOS, iNOS, and cNOS levels were categorized into low level (≤median) and high level (> median). A variable composed of the following four groups combining iNOS and cNOS levels was created: 1) low iNOS levels with low cNOS levels, 2) low iNOS levels with high cNOS levels, 3) high iNOS levels with low cNOS levels, and 4) high iNOS levels with high cNOS levels. Logistic regression models (adjusted age, BMI, marriage, and living status) were used to estimate whether CSF iNOS levels and CSF cNOS levels measures predict CSF tNOS levels. Binary logistic regression models (adjusted for age, BMI, marriage, and living status) were used to estimate the adjusted odds ratio (OR) of sleep disorders (PSQI scores ≥5) and six domains according to smoking and CSF NOS levels.

Furthermore, adjusting for age, BMI, marriage, and living companion status, a mediation effect analysis was performed to clarify whether CSF tNOS, iNOS, or cNOS mediated the relationship between smoking and PSQI global scores. The bootstrap sampling method was used to explore the effect decomposition from mediation models. Direct effect is the effect of an independent variable acting on a dependent variable after controlling for the mediating variable. After controlling for the mediating variables, the effects of the independent variables on the mediators and the effects of the mediators on the dependent variables constitute the indirect effects of the relationship between the variables. The total effect is equal to the direct effect plus the indirect effect, that is, the effect of the independent variable acting on the dependent variable when the mediating variable is not considered. All data were analyzed using the R programming language (version 4.2.2), and the significance level was set to 0.05.

Results

Sociodemographic and Clinical Characteristics

Table 1 presents our previously reported baseline clinical characteristics.[18] Non-smokers and active smokers differed significantly in age (34.4 ± 10.5 vs 29.6 ± 9.5, $p < 0.05$), sleep disorders (no or yes), marriage (married or unmarried), living status (low or high), BMI (25.9 ± 3.6 vs 24.9 ± 4.0, $p < 0.01$), and PSQI scores (4.3 ± 2.5 vs 2.9 ± 2.5, $p < 0.001$). Total NOS (14.7 ± 5.6 vs 28.3 ± 7.2, $p < 0.001$), iNOS (10.3 ± 2.7 vs 16.0 ± 5.4, $p < 0.001$), and cNOS (4.4 ± 3.9 vs 12.4 ± 6.9, $p < 0.001$) of active smokers were significantly lower than those of non-smokers.

Correlation Between NOS and PSQI Scores

Spearman correlation analysis was used to explore the relationship between NOS and PSQI scores (Table 2 and Supplementary Figure 1). CSF tNOS, iNOS, and cNOS were negatively correlated with PSQI scores ($p < 0.001$) in the general population, and the correlation coefficients ranged from −0.29 to −0.44.

Odds Ratios of Sleep Disorders and Six Domains According to Smoking and CSF NOS Level

Supplementary Table 1 shows the results of binary logistic regression analyses in which the presence of sleep disorders was predicted according to smoking and levels of CSF tNOS, iNOS, and cNOS. Individuals with high CSF iNOS levels (>median CSF iNOS level) had a lower prevalence of sleep disorders (OR: 0.40, 95% confidence interval [CI]: 0.17–0.92) compared to those with low CSF iNOS levels (≤median CSF iNOS level). Similarly, consistent results were observed for CSF cNOS levels, where individuals with high levels (>median CSF cNOS level) had a lower prevalence of sleep disorders (OR: 0.28, 95% confidence interval [CI]: 0.12–0.63) compared to those with low levels (≤median CSF cNOS level).

Table 1 Comparisons Between Non-Smokers and Active Smokers

Variable	Total (n=191) Mean ± SD	Non-Smokers (n=104) Mean ± SD	Active Smokers (n=87) Mean ± SD	*P*
Age, years	31.8±10.2	29.6±9.5	34.4±10.5	<0.05*
BMI, kg/m²	25.4±3.9	24.9±4.0	25.9±3.6	<0.01**
PSQI Global Score	3.5±2.6	2.9±2.5	4.3±2.5	<0.001***
SBP, mmHg	128.8±13.1	129.8±12.8	127.6±13.5	0.25
DBP, mmHg	76.0±10.5	75.2±9.4	76.9±11.6	0.30
HDL, mmol/L	1.3±0.3	1.3±0.3	1.2±0.3	0.36
LDL, mmol/L	2.7±0.7	2.7±0.8	2.7±0.6	0.89
TG, mmol/L	1.8±1.2	1.8±1.1	1.8±1.3	0.28
CHO, mmol/L	4.7±0.9	4.7±1.0	4.8±0.8	0.44
ALT, U/L	30.7±22.8	30.0±22.8	31.5±22.9	0.70
AST, U/L	21.1±8.5	21.5±9.4	20.6±7.4	0.66
GGT, U/L	43.4±38.1	40.4±30.8	47.3±45.6	0.43
CSF Total NOS, U/mL	21.2±9.4	28.3±7.2	14.7±5.6	<0.001***
CSF Inducible NOS, U/mL	15.9±8.6	16.0 ± 5.4	10.3 ± 2.7	<0.001***
CSF Constitutive NOS, U/mL	8.2±7.0	12.4 ± 6.9	4.4 ± 3.9	<0.001***
Age of smoking initiation	(-)	(-)	20.1 ±3.8	(-)
Total duration of smoking	(-)	(-)	14.1 ±9.3	(-)
Average number of cigarettes smoked	(-)	(-)	15.4 ±7.9	(-)
Maximum number of cigarettes smoked per day	(-)	(-)	18.5 ±9.0	(-)
	n (%)	n (%)	n (%)	
Sleep disorders				<0.05*
No	131 (69%)	79 (76%)	52 (60%)	
Yes	60 (31%)	25 (24%)	35 (40%)	
Marriage				<0.05*
Married	77 (40%)	52 (50%)	25 (29%)	
Unmarried	114 (60%)	52 (50%)	62 (71%)	
Living status				<0.05*
Living alone	44 (23%)	31 (30%)	13 (15%)	
Living with others	147 (77%)	73 (70%)	74 (85%)	

Notes: Marriage was tested using the chi-square test between non-smokers and active smokers, and other data were reported as Mann–Whitney sum tests between non-smokers and active smokers. *p < 0.05, **p < 0.01, ***p < 0.001.

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); PSQI, Pittsburgh Sleep Quality Index; SBP, systolic blood pressures; DBP, diastolic blood pressures; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; CHO, cholesterol; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; CSF, cerebral spinal fluid; NOS, nitric oxide synthase.

In addition, we used binary logistic regressions to further examine whether CSF tNOS level predicted sleep disorders and its six domains. The result of Supplementary Table 2 showed that CSF iNOS levels (OR: 1.59, 95% confidence interval [CI]: 1.39–1.87) and CSF cNOS levels (OR: 1.07, 95% confidence interval [CI]: 1.01–1.14) could predict tNOS levels. The results of logistic regressions showed that individuals with high levels of both iNOS and cNOS have a lower prevalence of sleep disorders (OR: 0.15, 95% confidence interval [CI]: 0.05–0.45) compared to those with low levels of both iNOS and cNOS (see Supplementary Table 3). Supplementary Figure 2 shows that CSF tNOS levels were not significantly associated with the six dimensions of sleep disorders.

Mediation Analysis

The mediation model is based on correlation analysis and Linear regression analysis. Further mediation analyses were conducted to evaluate the influence of CSF tNOS, iNOS, and cNOS between smoking and sleep quality. First, we examined the effect of cigarette smoking on PSQI scores after adjusting for age, BMI, marriage, and living status (see Table 3 and Figure 1); the linear regression analysis revealed that active smoking was associated with higher PSQI scores compared with nonsmoking ($R^2 = 0.1112$, $\beta = c = 0.109$, $t = 3.206$, $p < 0.001$). After adjusting for age, BMI, marriage, and living status, we

Table 2 Correlation of Physiological Indices with PSQI Global Scores in the General Group

	HDL	LDL	ALT	CHO	TG	GGT	AST	tNOS	iNOS	cNOS	PSQI
HDL	1***										
LDL	0.05	1***									
ALT	−0.13	0.14	1***								
CHO	0.26***	0.90***	0.15*	1***							
TG	−0.31***	0.35***	0.30***	0.35***	1***						
GGT	−0.19*	0.32***	0.55***	0.32***	0.42***	1***					
AST	−0.09	0.06	0.81***	0.09	0.22**	0.42***	1***				
tNOS	0.07	0.05	−0.05	0.03	0.07	−0.07	−0.004	1***			
iNOS	0.07	−0.03	0.03	−0.11	−0.02	−0.13	−0.003	0.46***	1***		
cNOS	0.03	0.02	0.004	−0.04	0.08	−0.10	0.03	0.81***	0.46***	1***	
PSQI	0.04	0.07	−0.08	0.12	−0.06	0.09	−0.14*	−0.29***	−0.37 ***	−0.44 ***	1***

Notes: All data were reported as Spearman correlation analyses. *p < 0.05, **p < 0.01, ***p < 0.001.
Abbreviations: PSQI, Pittsburgh Sleep Quality Index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; CHO, cholesterol; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; tNOS, total nitric oxide synthase; iNOS, inducible nitric oxide synthase; cNOS, constitutive nitric oxide synthase.

Table 3 Analysis of Smoking and PSQI Global Scores Association with CSF Total NOS as a Mediator

	Model1 (PSQI)		Model2 (tNOS)		Model3 (PSQI)	
	β	t	β	t	β	t
Age, y	0.126	1.250	0.024	0.304	0.130	1.307
BMI	0.155	1.685	0.037	0.506	0.162	1.776
Marriage	0.003	0.069	−0.026	−0.676	−0.002	−0.033
Living status	0.039	0.596	0.032	0.622	0.045	0.695
Smoke	0.109***	3.206	−0.451***	−16.773	0.023	0.428
CSF Total NOS	(–)	(–)	(–)	(–)	−0.191*	−2.072
R^2	0.111		0.623		0.132	
F (df)	4.65*** (5, 185)		61.10*** (5, 185)		4.66*** (5, 185)	

Notes: Model 1 was a linear regression model with smoke as the independent variable and PSQI as the dependent variable. Model 2 was a linear regression model with CSF Total NOS as the independent variable and PSQI as the dependent variable. Based on Model 1, Model 3 uses CSF total NOS and smoke as independent variables. All data were reported as mediation analyses. *p < 0.05, ***p < 0.001.
Abbreviations: PSQI, Pittsburgh Sleep Quality Index; BMI, body mass index; CSF, cerebral spinal fluid; tNOS, total nitric oxide synthase.

tested the effect of cigarette smoking on CSF tNOS (see Table 3 and Figure 1); there was a negative effect of tobacco on CSF tNOS ($R^2 = 0.623$, β =a1 =−0.451, t =−16.773, p < 0.001). Finally, we tested the mediating effect of cigarette smoking on PSQI scores by adding the mediator variables CSF tNOS (see Table 3 and Figure 1); the linear regression revealed a negative correlation between CSF tNOS level and PSQI score ($R^2 = 0.132$, β =b1 =−0.191, t =- 2.072, p < 0.05). The mediation analysis

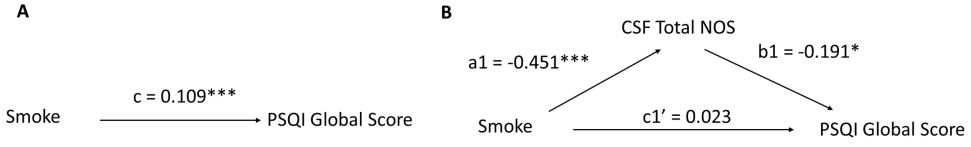

Figure 1 Effect decomposition of mediation models in PSQI global score.
Notes: (**A**) Total effect between smoking and PSQI global score. (**B**) Effect decomposition of the mediation model for the relationship between smoking and PSQI global score association with CSF Total NOS as mediator. *p < 0.05, ***p < 0.001.

Table 4 Significance Test for Mediating Effects of Smoking, tNOS, and PSQI Global Scores

Effect Decomposition	Estimated	95% CI		*P*
		Lower	Upper	
Indirect effect	0.086	0.003	0.170	0.042*
Direct effect	0.023	−0.080	0.130	0.654
Total effect	0.109	0.041	0.170	<0.001***

Notes: All data were reported as mediation analysis. Effect decomposition of the mediation model for the relationship between smoking and PSQI global score association with CSF Total NOS as mediator. *p < 0.05, ***p < 0.001.
Abbreviation: 95% CI, 95% confidence interval.

(as shown in Table 4 and Figure 1) revealed a significant mediating effect of CSF tNOS in the relationship between smoking and PSQI Scores (indirect effect: z=a1*b1 = 0.086, 95% CI [0.003–0.170], p < 0.05; direct effect: z = c1'= 0.023, 95% CI [−0.080–0.130], p = 0.654; total effect: z = c = 0.109, 95% CI [0.041–0.170], p <0.001).

We also examined the mediating effects of iNOS and cNOS on the relationship between smoking and PSQI scores. As shown in Figure 2A, Supplementary Table 4 and Supplementary Table 5, there was a significant mediating effect of CSF iNOS on the relationship between cigarette smoking and PSQI scores (indirect effect: z = a2*b2= 0.073, 95% CI [0.031–0.120], p <0.001; direct effect: z =c2' = 0.037, 95% CI [−0.040–0.110], p = 0.341; total effect: z =c = 0.109, 95% CI [0.041–0.170], p <0.001). Similarly, CSF cNOS (indirect effect: z = a3*b3 = 0.089, 95% CI [0.046–0.140], p <0.001; direct effect: z =c3' = 0.019, 95% CI [−0.055–0.090], p = 0.608; total effect: z = c = 0.109, 95% CI [0.041–0.170], p <0.001) significantly mediated the relationship between cigarette smoking and PSQI scores (see Figure 2B, Supplementary Table 6 and Supplementary Table 7).

These findings suggest that the mediating effect of tNOS, iNOS, and cNOS was complete, and the ratio of mediating effect (tNOS, iNOS, and cNOS) to the total impact were 78.93%, 66.29%, and 81.65%, respectively.

Discussion

There was a positive correlation between cigarette smoking and PSQI scores. Significantly lower CSF levels of tNOS, iNOS, and cNOS were found in active smokers. This result was reported in our previous study.[18] Furthermore, tNOS, iNOS, and cNOS positively correlated with PSQI scores in the general group. A mediation analysis showed that tNOS, iNOS, and cNOS in CSF mediated the relationship between cigarette smoking and sleep quality measured by PSQI scores. To our knowledge, this is the first study to investigate this relationship. Our findings suggest that NOS in CSF may be a critical factor linking cigarette smoking to sleep quality.

A noteworthy observation was the negative correlation between CSF tNOS, iNOS, and cNOS levels and PSQI scores. NO produced by neuronal NOS is a potent sleep aid.[22] Multiple animal experiments have shown that nNOS knockout mice have less REM and non-REM sleep time and seizure frequency than normal mice.[23,24] Nevertheless, the relationship between iNOS and eNOS and sleep disorders is controversial. Mice lacking the iNOS gene have a longer REM time than normal mice.[25] However, some studies indicated that the iNOS expression ensures adequate maintenance of REM sleep with aging.[11] Another study found that the basal forebrain of AD patients had higher levels of iNOS and NO, an essential mechanism for maintaining sleep homeostasis.[10,22,26] In addition, the corpus cavernosum

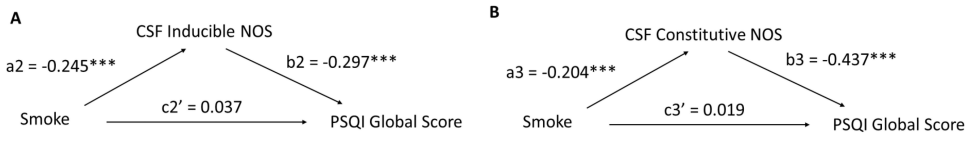

Figure 2 Effect decomposition of mediation models in the PSQI global score.
Notes: (**A**) Effect decomposition of the mediation model for the relationship between smoking and PSQI global score association with CSF Inducible NOS as mediator. (**B**) Effect decomposition of the mediation model for the relationship between smoking and PSQI global score association with CSF Constitutive NOS as mediator. ***p < 0.001.

tissue of sleep-deprived rats has higher eNOS levels than normal rats.[27] The role of iNOS and eNOS in sleep regulation requires further investigation.[28] Our study focused on CSF NOS, providing an addition to the field of sleep research.

Interestingly, the mediation analysis revealed that CSF tNOS, iNOS, and cNOS levels had a mediating effect on increased PSQI scores due to cigarette smoke exposure, and the indirect effect accounted for 78.93%, 66.29%, and 81.65% of the total effect. Studies have suggested nicotine may stimulate neurotransmitters involved in sleep-wake cycle regulation, including dopamine, norepinephrine, and γ-aminobutyric acid, disrupting sleep architecture.[29,30] Other studies showed that chemicals in cigarette smoke may induce sleep disorders by increasing the inflammatory response in the upper respiratory tract.[31,32] However, there are no reports on the role of CSF NOS content in the relationship between smoking and sleep quality. Therefore, our study provides clues for CSF NOS as a potential mediator between cigarette smoking and sleep quality.

The physiological mechanisms underlying the relationship between cigarette smoking and sleep disorders are unclear.[33] Some scholars speculate that nicotine induces alterations in brain neurochemistry or disruptions to development, leading to sleep behavior disturbances.[30,33] It is worth noting that toxic chemicals in cigarettes induce the generation of free radicals and a decrease in NOS and endogenous NO.[8,9,21] The NOS family catalyzes the conversion of L-arginine to NO and citrulline.[11] NO is a neurotransmitter and neuromodulator involved in sleep regulation.[11,34] Kapa et al demonstrated that increasing NO levels in the brain promoted spontaneous sleep in rats.[35] NO may function as a signal molecule in the steady-state regulation of slow-wave sleep.[36] NO affects REM sleep by regulating acetylcholine, γ-aminobutyric acid, serotonin, and adenosine levels.[37] In addition, smokers have less basal endogenous nitric oxide synthesis in their airways and blood vessels than ordinary people. Subsequently, smokers may experience airway constriction due to nitric oxide's involvement in maintaining airway dilation.[38] This is likely one of the mechanisms through which smoking causes sleep apnea syndrome.[37,39,40] Interestingly, NO produced by NOS can also inhibit the release of inflammatory mediators.[39] Sleep disorders and inflammatory responses are related.[41] IL-6 levels significantly increase in patients with insomnia, and IL-6 can spread in the periventricular area, sending immune signals to the brain that affect sleep-related processes.[41]

There are several important research implications. Firstly, they emphasize the importance of considering the levels of CSF NOS when assessing the relationship between smoking and sleep quality. Increasing NOS levels in smokers may improve sleep quality. Clinicians may need to monitor the levels of NOS in smokers reporting poor sleep quality and consider targeted interventions aimed at increasing NOS levels, such as supplementation with antioxidant vitamins.[42] Secondly, the study results suggest that NOS may be one of the biological mechanisms underlying the relationship between smoking and sleep quality. This suggests that modulating NOS levels may be a potential target for improving sleep quality in smokers. Overall, our study contributes to understanding the complex interactions between smoking, NOS, and sleep quality.

Limitations

There were some limitations to the present study. First, the participants were patients with anterior cruciate ligament injury, which may confound our results. Second, studies have shown that women and men who undergo surgery differ significantly in many postoperative reactions, including postoperative pain and postoperative sleep.[43,44] However, we only collected data on male smokers, which may have led to neglecting gender factors in the discussion. The investigation of gender differences may be a key direction for future research. Third, our study had a relatively small sample size (n = 191), with a minority of individuals experiencing sleep disorders (n = 60, 31.4%). In future research, we intend to increase the sample size to explore further the relationship between smoking, NOS, and sleep. Finally, we only dealt with a single aspect of NOS: its content. It acts via different mechanisms in inflammatory and immune diseases, insulin resistance, and tumors. NOS activity is also essential, as it provides direction for future research.

Conclusions

This study found that cigarette smoking is associated with sleep disorders, and tNOS, iNOS, and cNOS in CSF mediate the relationship between smoking and sleep quality. Protective Effects of CSF tNOS, iNOS, and cNOS provide new

therapeutic targets for improving sleep quality. However, the specific relationship between smoking and sleep disorders needs to be further validated and explained in vivo and in vitro studies with larger sample sizes.

Abbreviations

NOS, nitric oxide synthase; CSF, cerebrospinal fluid; tNOS, total NOS; iNOS, inducible NOS; cNOS, constitutive NOS; PSQI, Pittsburgh Sleep Quality Index; eNOS, endothelial NOS; NO, nitric oxide; REM, rapid eye movement; BMI, body mass index; SBP, systolic blood pressures; DBP, diastolic blood pressures; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; CHO, cholesterol; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase.

Inclusion of Identifiable Human Data

No potentially identifiable human images or data is presented in this study.

Studies Involving Animal Subjects

Generated Statement: No animal studies are presented in this manuscript.

Studies Involving Human Subjects

The present study was approved by the Institutional Review Board of Inner Mongolian Medical University, and was performed in accordance with the Declaration of Helsinki. And there was no financial compensation provided to the subjects. The patients/participants provided their written informed consent to participate in this study. And there was no financial compensation provided to the subjects.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author, Yanlong Liu, on reasonable request.

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Author Contributions

All of our authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; At the same time, all authors have gave final approval of the version to be published and have agreed on the journal to which the article has been submitted. In addition, all authors have reviewed and agreed on all versions of the article prior to submission, during the revision process, acceptance of the final version for publication, and any significant changes introduced during the proofreading phase. All authors have agreed to take responsibility and responsibility for the content of the article.

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Disclosure

The authors declare that they have no competing interests in this work.

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