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# Molecular Interactions between Gasotransmitters in Patients with Obstructive Sleep Apnea

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**Abstract:** Patients with obstructive sleep apnea (OSA) have an increased risk of cardiovascular disease (CVD). Nitric oxide (NO) and heme oxygenase-1 (HO-1) affect vascular tone and are vasoprotective. Furthermore, hydrogen sulfide ($H_2S$), an HO-1 inducer, is known to be a major effector molecule driving apneas. This study was conducted to examine the molecular relationships between these gasotransmitters and HO-1 in patients with OSA. Individuals who presented for evaluation for possible OSA were recruited and underwent overnight polysomnography. Individuals with an apnea-hypopnea index (AHI) of >5 per hour (OSA diagnosis) were considered cases ($n$ = 19), while those with an AHI of <5 per hour ($n$ = 6) were the controls. Blood samples were obtained before sleep and again from OSA cases prior to initiating treatment. $H_2S$, NO, and HO-1 levels were assayed. Patients with OSA showed lower NO and $H_2S$ levels at baseline compared to controls. NO levels further decreased significantly from baseline in patients at the time of OSA diagnosis, while $H_2S$ levels largely showed an increasing trend, which was observed only when the subjects showing a baseline $H_2S$ level of >0.5 µM were excluded. Interestingly, analysis of HO-1 did not show a significant change from baseline, confirming the inverse relationship between the two gasotransmitters. The alterations in the bioavailability of endogenous $H_2S$ and its molecular interactions with NO and HO-1 regulating vascular tone may play a role in the pathogenesis of CVD in OSA patients.

**Keywords:** hydrogen sulfide ($H_2S$); nitric oxide (NO); heme oxygenase-1 (HO-1); obstructive sleep apnea (OSA); cardiovascular disease (CVD)

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## 1. Introduction

Obstructive sleep apnea (OSA) and cardiovascular disease (CVD) are common co-morbidities. The estimated prevalence of OSA at AHI >= 15 events per hour ranges from 6 to 17% in the general population, up to 49% in older subjects and patients with established CVD [1]. Indeed, OSA has been implicated as an independent risk factor for CVD [2–6]. Endothelial dysfunction is one of the likely mechanisms contributing to the heightened CVD risk in OSA patients, in addition to other factors such as increased sympathetic activity, oxidative stress, and inflammation [7–9]. According to the World Health Organization (WHO), in 2016 almost 18 million deaths were related to CVD [10]. As a step towards reducing the risk of CVD in OSA patients, the underlying pathophysiological mechanisms linking CVD and OSA must be better elucidated [11].

Nitric oxide (NO) and heme oxygenase-1 (HO-1) have both been shown to modulate vascular tone [12]. Both NO and NO derivatives are potent stimulators of HO-1 production [13]. Reduced endothelial nitric oxide synthase (eNOS) expression leading to decreased NO levels is one of the earliest changes implicated in endothelial dysfunction [14,15]. This feature is consistently noted in patients with OSA and is an important predictor of

atherosclerosis leading to CVD [6]. HO-1 is an anti-atherogenic enzyme that may be a promising therapeutic target in the treatment of vascular disease, due to its vasoprotective effects [16]. End products of HO-1 include a second gasotransmitter, carbon monoxide (CO), and the bile pigments bilirubin and biliverdin, which have anti-inflammatory, anti-oxidant, anti-apoptotic, and antithrombotic effects [17–19]. Overexpression of HO-1 in animal models has been shown to restore eNOS, increase the production of NO, and improve the endothelium-dependent vascular relaxation [20]. In contrast, a reduction in CO caused increased sleep apnea indices during rapid eye movement (REM) sleep [21]. Treating OSA increases endogenous levels of NO, potentially improving cardiovascular outcomes [22].

Hydrogen sulfide ($H_2S$) is another gasotransmitter that has recently gained importance due to its anti-oxidative and cytoprotective properties and its ability to induce HO-1 [23,24]. $H_2S$ plays a potential role in several physiological mechanisms, including endothelial function, vasomotor tone regulation, and angiogenesis, which are implicated in CVD [25,26]. $H_2S$ is shown to have close interactions with the NO pathway that could potentially affect the endothelial function [27]. However, $H_2S$ is shown to have both pro-inflammatory and anti-inflammatory actions. $H_2S$ can cause either vasodilation or vasoconstriction in a concentration-dependent manner, thereby exerting a biphasic effect on the vascular endothelium [28]. Higher concentrations of $H_2S$ in the presence of NO can induce significant vasodilation by opening $K_{ATP}$ channels, while this effect is attenuated with a reduction in NO levels. At lower concentrations, $H_2S$ has been shown to inhibit endothelial nitric oxide synthase (e-NOS), causing vasoconstriction [29].

Very little is known about the bioavailability of $H_2S$ in the context of OSA. Moreover, no studies have examined the molecular interaction of these gasotransmitters, specifically with respect to HO-1. We speculate that alterations in the bioavailability of endogenous $H_2S$ and the consequent adaptations in the molecular interactions between $H_2S$, NO, and HO-1 regulating the vascular tone may play a role in the pathogenesis of CVD in OSA patients. This preliminary study was designed to examine such alterations in newly diagnosed OSA patients, with a long-term goal of not only gaining insights into the pathophysiological mechanisms leading to CVD but also developing clinically effective therapeutic modalities that target these gasotransmitters to reduce the CVD risk in these patients.

## 2. Methods

The study was approved by the Institutional Review Board at the University of Minnesota. Informed written consent was obtained from all the study subjects. The recruitment took place from September 2012 through April 2013.

### *2.1. Study Participants*

A total of twenty-five patients who were referred to our academic sleep center for evaluation for possible OSA were recruited. The inclusion criteria included no previous diagnosis of OSA, naïve to CPAP treatment, ages 25–75 years, with BMI greater than 30 $kg/m^2$. Patients using corticosteroids, oral contraceptives, and anti-inflammatory agents, and those with chronic obstructive pulmonary disease, neuromuscular disorders, recent or concomitant systemic infections, or upper or lower airway infections and autoimmune diseases were excluded. History, physical exam results, blood pressure measurements, and overnight polysomnography (PSG) were obtained for all subjects. The cases and controls were identified during the PSG, based on the apnea-hypopnea index (AHI), which is the average number of episodes of apneas and hypopneas per hour of sleep. The cases were subjects who qualified for the diagnosis of OSA with an AHI of more than 5 per hour ($n$ = 19), while those without OSA, with an AHI of <5 per hour ($n$ = 6), acted as controls.

### *2.2. Polysomnography (PSG)*

All subjects underwent supervised overnight PSG for evaluation of OSA at the University of Minnesota Sleep Center. The PSG monitoring system (Excel-Tech Corp., Oakville, ON, Canada) included techniques for measuring standard physiologic parameters, such as electroencephalog-

raphy, electrooculography, electromyography, electrocardiography, a thermistor nasal pressure transducer and instrumentation for oral airflow measurements. The respiratory parameters of chest and abdominal movements were recorded using respiratory inductance plethysmography. Oxygen saturation was recorded using pulse oximetry. All studies were manually scored by an experienced registered PSG technologist and were reviewed and interpreted by a board-certified sleep physician. Disordered breathing events were scored in accordance with guidelines from the American Academy of Sleep Medicine [30].

### *2.3. Sample Collection*

Approximately 4 mL of venous blood was collected from each subject in an EDTA tube and a sodium heparin tube. Initial baseline blood samples were obtained from all the subjects before sleep. A second blood sample was obtained only from patients diagnosed with OSA, prior to initiation of treatment with continuous positive airway pressure (CPAP). CPAP was initiated if the AHI was greater than 10 per hour. The blood was processed immediately to separate the plasma in the sleep lab. Blood collected in heparin tubes was centrifuged immediately at 3000 rpm for 2 min to separate the plasma, and ~300 µL of plasma was added to the stabilizing solution provided for the $H_2S$ assay. All samples were frozen until analysis.

### *2.4. $H_2S$ Analysis*

$H_2S$ analysis was performed using a modified methylene blue reaction coupled with high-performance liquid chromatography (HPLC) detection with slight modifications [31]. Shortly after collection, 0.5 mL of plasma was added to a stabilizing solution consisting of 350 µL of 1% zinc acetate and 50 µL of 1.5 M sodium hydroxide. The samples were then frozen at −80 °C until analysis. A 6-point standard curve (0.1–3 µM) was generated from an $H_2S$ stock solution (100 µM) that was prepared by spiking phosphate-buffered saline (PBS) with 1 mM sodium hydrogen sulfide (NaHS; Alfa Aesar, MA, USA) and processed identically to the plasma samples. The methylene blue color development was achieved by reaction with 20 mM N, N-dimethyl-p-phenylenediamine dihydrochloride (N,N-dpd; Sigma-Aldrich, St. Louis, MO, USA) in 7.2 N HCl and 30 mM ferric chloride (Fisher Scientific), in 1.2 N HCl. After separation of protein with 10% trichloroacetic acid (Ricca Chemical Company, Arlington, TX, USA), the clear supernatant was taken for HPLC analysis, performed in a series 1260 Infinity system (Agilent Technologies, Santa Clara, CA, USA) connected to a UV/Vis detector to measure absorbance at 667 nm. Peak separation was achieved using a mobile phase consisting of a 72% solution containing 0.15% $v/v$ trifluoroacetic acid (Sigma-Aldrich) in ultra-pure water and 28% acetonitrile, using an Eclipse XDB-C18 column. The HPLC system was controlled, and data were processed, using ChemStation software (Agilent Technologies, Santa Clara, CA, USA). The retention time was observed to be ~5.6 min. GraphPad Prism 4 (GraphPad Software, Inc., San Diego, CA, USA) was used to create a standard curve and perform a nonlinear second-order polynomial (quadratic) regression. Acceptable $r^2$ values were $\geq$0.995.

### *2.5. Nitric Oxide Analysis*

Ultra-filtered plasma samples were analyzed using a nitrate/nitrite colorimetric assay kit (Cayman Chemicals, Ann Arbor, MI, USA) as per the manufacturer's instructions. The sum of nitrates and nitrites, which are stable end products of NO metabolism, is considered an index of total NO production. Absorbance was measured using a Synergy™ 2 multi-detection microplate reader (BioTek, Winooski, VT, USA). The concentration of nitrates + nitrites in the patient samples was determined by plotting the absorbance readings as a function of NO concentration and performing a linear regression analysis. Values from the regression were substituted into the formula in the assay protocol to calculate the total NO (nitrate + nitrites in µM) in the sample. The lower limit of detection was 5 µM.

### *2.6. Heme Oxygenase-1 (HO-1) Analysis*

Plasma HO-1 levels were determined using the enzyme-linked immunosorbent assay (ELISA) method (HO-1 [human] ELISA kit, Enzo Life Sciences, Farmingdale, NY, USA) as per the manufacturer's protocol and as described previously [32].

### *2.7. Biostatistical Analysis*

Descriptive statistics were shown as frequencies and percentages or as the mean ± standard error (SE), as appropriate. For continuous variables, *p*-values were calculated using a two-sample *t*-test or a Wilcoxon rank test, depending on the distribution of data for two-group comparison, a Wilcoxon signed rank test for paired comparison, or a Kruskal–Wallis test for three-group comparison. For categorical variables, the *p*-value was calculated using Fisher's exact test. For bivariate association, Spearman's correlation coefficient was calculated. All analyses were performed using the SAS system (v. 9.3; SAS Institute, Cary, NC, USA). The *p*-values were two-sided, and $p < 0.05$ was considered statistically significant.

## 3. Results

### *3.1. Participant Characteristics*

The baseline characteristics of all participants are shown in Table 1. Of the 25 subjects recruited, we identified 19 patients with OSA (11 males and 8 females) and 6 controls without OSA (1 male and 5 females). Patients with OSA were older compared to controls, 51 ± 12 vs. 36 ± 11 years, respectively. BMI, height, and smoking history (current or former) were similar in both groups. The prevalence of medical comorbidities, including hypertension, diabetes mellitus, coronary artery disease, congestive heart failure, hyperlipidemia, and atrial fibrillation were also similar in both groups.

**Table 1.** Demographic and clinical characteristics of participants in this study.

| Variable | Cases<br>*n* = 19<br>Mean ± SE or Number | Control<br>*n* = 6<br>Mean ± SE or Number | *p*-Value |
|---|---|---|---|
| **Age (Years)** | 36.2 (4.8) | 51 (2.7) | 0.0127 |
| **Gender, Male** | 11 (57.9) | 1 (16.7) | 0.0780 |
| **BMI (Kg/m$^2$)** | 38.5 (1.3) | 36.8 (2.3) | 0.53 |
| **Height (Meters)** | 1.73 (0.02) | 1.64 (0.04) | 0.06 |
| **Hypertension** | 10 | 2 | 0.4095 |
| **Diabetes Mellitus** | 5 | 1 | 0.6295 |
| **Congestive Heart Failure** | 1 | 1 | 0.3694 |
| **Coronary Artery Disease** | 3 | 2 | 0.3490 |
| **Atrial Fibrillation** | 1 | 0 | 0.5663 |
| **Hyperlipidemia** | 7 | 2 | 0.8760 |
| **Current or Former Smoker** | 11 | 1 | 0.0780 |

### *3.2. Alterations in Plasma Gasotransmitter Levels following Obstructive Events*

Baseline levels of both NO and $H_2S$ in patients with OSA were lower compared to controls, though there were no statistically significant differences (Table 2). The trend towards reduction of these gasotransmitters in OSA patients is evident when we calculate the ratio of $H_2S$ to the total NO products ($H_2S$/NO). Notably, the baseline concentration of HO-1 was identical in both OSA patients and controls.

**Table 2.** Baseline levels of the gasotransmitters and HO-1 in samples collected prior to sleep. All data are represented as mean (SE).

| Variable | OSA ($n$ = 19) | Control ($n$ = 6) | $p$-Value |
|---|---|---|---|
| NO (measured as nitrites and nitrites in μM) | 32.28 (16.16) | 38.30 (34.00) | 0.9749 |
| $H_2S$ (in μM) | 0.25(0.21) | 0.30 (0.25) | 0.6153 |
| $H_2S$/NO ratio | 9.88 (9.33) | 13.40 (16.80) | 0.9749 |
| HO-1 (ng/mL) | 2.92 (1.38) | 2.96 (1.89) | 0.9749 |

Subsequent analysis of the samples collected prior to the initiation of CPAP treatment in patients with OSA ($n$ = 17) revealed a significant decrease in NO from the baseline mean levels after an average of 233 ± 60.5 min of untreated OSA. The mean reduction in NO was −3.6 μM ± 6.73 ($p$ = 0.03). In contrast, we did not observe a clear difference in the levels of $H_2S$ between the two time points. However, it was interesting to note that when we excluded subjects with a baseline $H_2S$ level of >0.5 μM ($n$ = 4), we observed a trend towards an increase in $H_2S$ levels from baseline to the time of diagnosis of OSA. The average increase in $H_2S$ levels was observed to be 0.125 ± 0.06 μM ($p$ = 0.09) after obstructive events (Figure 1). We sought to understand if there were any differences in the co-variables among these four cases with high baseline $H_2S$. Three out of the four cases had an AHI greater than 70 per hour but did not have REM sleep during the baseline. Two of the four cases showed evidence of sleep-associated hypoxemia, which is defined as time spent with oxygen saturation at or below 88% for more than 5 min [33], with no REM, in the baseline study. There were no other differences in the co-variables tested among the four cases.

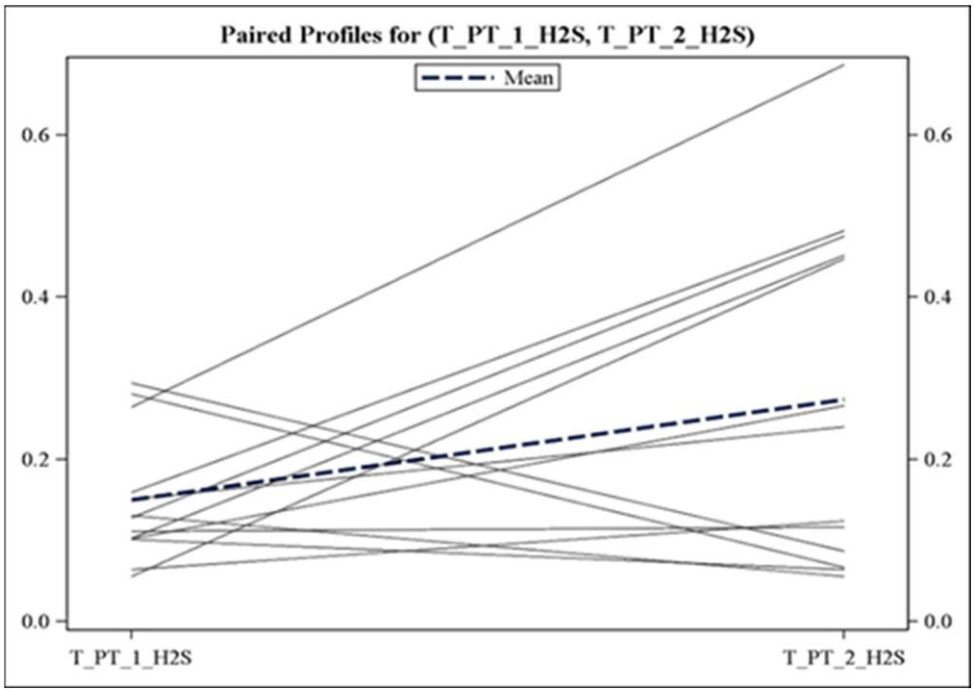



**Figure 1.** Individual trajectories of $H_2S$ concentration in patients with OSA. $H_2S$ levels (in μM) were analyzed at baseline (before sleep, T_PT_1) and at the time of OSA diagnosis (T_PT_2). Data from patients with baseline $H_2S$ of >0.5 μM ($n$ = 4) were not included.

### *3.3. Effect of Obstructive Events on Heme Oxygenase-1 (HO-1) Expression*

In addition to the gaseous mediators, we measured the concentration of the antioxidant protein HO-1 in plasma samples. HO-1 mRNA is known to be induced both by NO and $H_2S$ [34].

However, we did not observe any difference in HO-1 levels in samples collected from 17 patients with OSA after obstructive events, compared to the mean baseline concentra-

tions. The mean concentration of HO-1 at the second time point, prior to the initiation of CPAP treatment, was 2.92 ± 1.46 ng/mL. We further confirmed the relationship between HO-1 protein concentrations and the two gasotransmitter levels at the time of OSA diagnosis. Our analysis showed significant negative (r = −0.495, $p = 0.04$) and positive (r = 0.458, $p = 0.06$) correlations between HO-1 and NO and $H_2S$, respectively, confirming the inverse relationship between the two gasotransmitters.

### *3.4. Effect of Severity of OSA on Baseline Levels of NO, $H_2S$, and HO-1*

To further understand the interactions between these molecules, we compared baseline levels of NO, $H_2S$, and HO-1 among patients with OSA in terms of OSA severity. The severity of OSA was classified based on the AHI into: mild OSA, if the AHI was >5 to <15 per hour, moderate OSA if the AHI was >15 to <30 per hour, and severe OSA (>30 per hour) [35]. Out of the 19 cases, there were 4 cases with mild OSA, 6 with moderate OSA, and 9 with severe OSA. NO levels were inversely proportional to the degree of severity of OSA, similarly to previous observations [36]. A similar analysis with baseline levels of $H_2S$ did not show a relationship with the degree of severity, probably due to higher variability in these values (Figure 2). Additionally, baseline HO-1 also showed no relationship with severity. However, correlation analysis of baseline NO and $H_2S$ levels revealed a moderate inverse relationship between these gasotransmitters (Spearman correlation coefficient r = −0.32; $p = 0.11$).

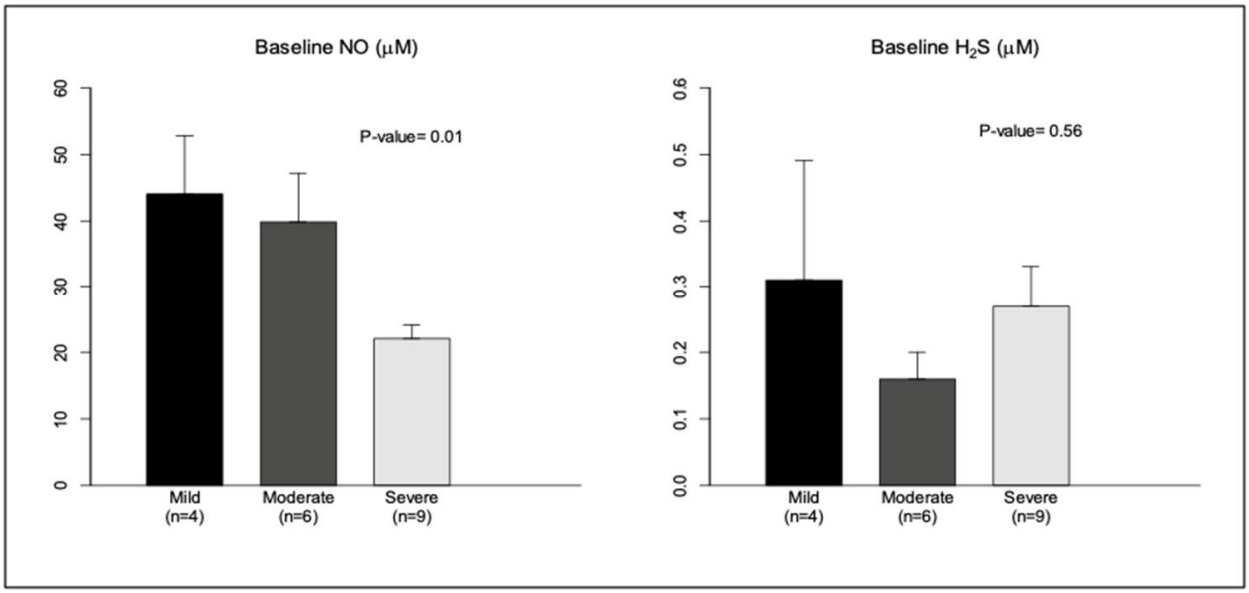



**Figure 2.** Baseline NO and $H_2S$ levels (in μM) were analyzed in relation to severity of OSA (AHI > 5/h). 1: Mild OSA (AHI 5–15/h); 2: moderate OSA (AHI 15–30/h); 3: severe OSA (AHI > 30/h). Data are shown as mean ± SE.

### *3.5. A Trend towards an Increase in $H_2S$ and Decrease in HO-1 with Frequent Periodic Limb Movements*

Periodic limb movements during sleep (PLMs) are episodes of periodic repetitive limb movements, commonly involving the lower extremities. PLMs and OSA have been associated with systemic inflammation and CVD. We categorized the patients based on the frequency of periodic limb movements index (PLMI) into two groups: those with a PLMI of >20 and those with a PLMI of <20 per hour. Of the 17 cases with OSA, 5 had a PLMI of >20 per hour.

Subjects with PLMI > 20 showed higher NO and lower $H_2S$ levels compared to subjects with PLMI < 20. When we analyzed the changes in gasotransmitter levels between the two time points, we found that NO levels largely decreased in all OSA patients, irrespective of the PLMI (data not shown). However, OSA patients with a PLMI of >20 per hour

showed an increase in $H_2S$ levels, and a decrease was observed in subjects with a PLMI of <20 per hour (0.20 ± 0.12 μM vs. −0.08 ± 0.08 μM; $p = 0.08$). On the other hand, we observed an inverse relationship between changes in $H_2S$ and HO-1 levels vs. PLM frequency (Figure 3). HO-1 levels decreased in subjects with a PLMI of >20 per hour and increased in those with a PLMI of <20 per hour (−0.64 ± 0.23 ng/mL vs. 0.14 ± 0.23 ng/mL; $p = 0.08$).

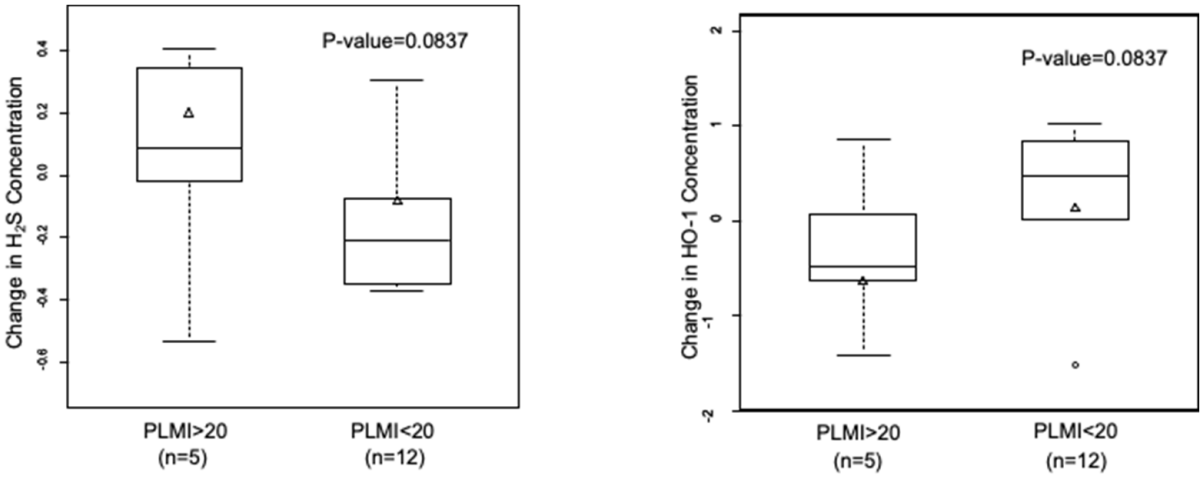



**Figure 3.** Inverse correlation in changes in $H_2S$ and HO-1 levels in relation to PLMI. Subjects with OSA diagnosis ($n = 17$) were grouped based on PLMI. Data are shown as mean ± SE.

## 4. Discussion

The gasotransmitters $H_2S$ and NO and the mediator protein HO-1 all play a role in regulating the vascular tone. To the best of our knowledge, this is the first report of the molecular interactions between plasma $H_2S$, NO, and HO-1 in OSA patients. Previously, Peng et al. demonstrated the complementary roles of CO and $H_2S$ in sleep apnea [21]. We demonstrated that for patients with OSA who have a baseline $H_2S$ concentration of <0.5 μM, there is a trend towards increasing its levels after a sufficient number of obstructive events. Furthermore, we showed that the functional consequence of the changes in gasotransmitter levels on downstream signaling is dependent on the relative magnitude of these alterations. Although HO-1 is a known downstream mediator protein of gasotransmitters, we showed that the level of this antioxidant is not altered in this cohort, probably due to the inverse correlation in $H_2S$ and NO activity.

OSA patients have recurrent episodes of hypoxemia followed by re-oxygenation, which can potentially cause rapid fluctuations in the levels of the gasotransmitters. Previous studies have shown that hypoxia induces $H_2S$ production [37]. It has also been shown that $H_2S$ enhances the sensory response of the carotid body to hypoxia, and the chemo-reflex mediates increased sympathetic nerve activity [38,39], supporting the potential role of $H_2S$ in CVD risk in OSA patients. Mice deficient in HO-2, an isoform of HO-1 responsible for CO production, showed carotid body hyperactivity and sleep apnea, which was normalized by genetic ablation of an $H_2S$-producing enzyme [21]. This indicates that $H_2S$ is a major effector molecule driving apneas. We observed a trend towards an increase in the $H_2S$ levels in OSA patients. It is plausible that there may have been a significant increase in $H_2S$ levels immediately after the apnea episodes, but this would have been transient, with the levels becoming reduced with the resumption of normoxia. This can be proved only by measuring oxygen and $H_2S$ in real time, which can be challenging in a sleep laboratory setting.

Previous studies have demonstrated the molecular interactions of $H_2S$ and NO [40]. King et al. recently demonstrated that the cardioprotective action of $H_2S$ is mediated by the activation of the enzyme endothelial nitric oxide synthase (e-NOS), which catalyzes the conversion of L-arginine to NO [28]. However, we observed an inverse relationship between NO and $H_2S$, which is in line with a recent report on plasma-free $H_2S$ levels in CVD [41].

The increase in $H_2S$, especially in those with low baseline $H_2S$ levels, could also be an endogenous countermeasure in response to decreased NO levels, aimed at maintaining HO-1 levels at equilibrium. Additionally, NO levels were observed to be inversely correlated with the severity of OSA, as previously reported [42]. A similar analysis with baseline levels of $H_2S$ or HO-1 did not show any correlation with the degree of severity. This is likely to be due to potential skewing by one subject with high $H_2S$ (0.8) but with mild OSA (AHI = 14 per hour) affecting the trend in $H_2S$ levels.

We also stratified the subjects based on periodic limb movements (PLMs). PLMs are more prevalent in OSA patients compared to the general population and have been associated with systemic inflammation and CVD [43]. In our study, patients with a high PLM index showed a trend towards an increase in $H_2S$ levels and a decrease in levels of NO and HO-1. Moreover, we observed an inverse correlation between baseline NO and $H_2S$ levels vs. PLM frequency, indicating that the effect of PLMs on gasotransmitters is one of the potential mechanisms by which PLMs could increase the risk of CVD in OSA patients.

## 5. Limitations

This was a preliminary study investigating plasma $H_2S$ levels in patients with OSA. We acknowledge several limitations in this study. The most important limitation is the small sample size and lack of a third time point after CPAP treatment. In addition, although the patients with OSA and the controls were similar with respect to gender, BMI, height, and medical comorbidities, the patients with OSA were approximately 15 years older. Enrolling patients with severe OSA (AHI > 30 per hour) and including a third time point for venous blood sampling post CPAP treatment to analyze the effects of CPAP on levels of $H_2S$, NO, and HO-1 would have strengthened our study. Lastly, screening for dietary factors such as garlic-containing diets and other herbal supplements may have been beneficial, since garlic-rich diets have been shown to increase the biological production of $H_2S$ [44]. However, in this preliminary investigation, we found that untreated OSA acutely lowers NO levels without concomitant reductions in HO-1 and a transient increase in $H_2S$. Further studies with larger sample sizes, including the effects of CPAP on $H_2S$, NO, and HO-1 in subjects with severe OSA, would be likely to provide further important information regarding the implications of these molecules and their interactions, leading to a better understanding of their implications for CVD risk in OSA patients.

## 6. Conclusions

Observations from our study showed that $H_2S$ could be a potential mediator contributing to CVD risk in OSA patients. Further studies with larger sample sizes, including subjects with severe hypoxemia, with measurements at various time points to understand the circadian variation, could elucidate the role of $H_2S$ in OSA.

**Author Contributions:** S.P. and R.V.K. designed the experiments; S.P., R.V.K., L.B.H. and J.L. performed the experiments; S.P., R.V.K., L.B.H., J.L., L.Z. and A.A. analyzed the data; R.V.K. contributed essential reagents/tools; S.P., R.V.K., L.B.H., J.L., L.Z. and A.A. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the University of Minnesota (protocol #1208M18662 approved on 6 December 2014).

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**Conflicts of Interest:** The authors declare no conflict of interest.

**Competing Financial Interests:** The authors declare no competing financial interests.

## Abbreviations

| | |
|---|---|
| OSA | Obstructive sleep apnea |
| CVD | Cardiovascular disease |
| NO | Nitric oxide |
| $H_2S$ | Hydrogen sulfide |
| 3-MST | 3-mercaptopyruvate sulfurtransferase |
| e-NOS | Endothelial nitric oxide synthase |
| CPAP | Continuous positive airway pressure |
| PSG | Polysomnography |
| AHI | Apnea-hypopnea index |
| AASM | American Academy of Sleep Medicine |
| HPLC | High-performance liquid chromatography |
| CHF | Congestive heart failure |
| CAD | Coronary artery disease |
| Afib | Atrial fibrillation |
| REM | Rapid eye movement |
| PLMs | Periodic limb movements during sleep |
| PLMI | Periodic limb movement index |
| ROS | Reactive oxygen species |

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