CLINICAL SCIENCE

EFFECTS OF SILDENAFIL ON AUTONOMIC NERVOUS FUNCTION DURING SLEEP IN OBSTRUCTIVE SLEEP APNEA

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OBJECTIVE: To evaluate the effects of sildenafil on the autonomic nervous system in patients with severe obstructive sleep apnea.
METHODS: Thirteen male patients with severe obstructive sleep apnea (mean age 43±10 years with a mean body mass index of 26.7±1.9 kg/m^2) received a single 50-mg dose of sildenafil or a placebo at bedtime. All-night polysomnography and heart rate variability were recorded. Frequency domain analysis of heart rate variability was performed for the central five-minute sample of the longest uninterrupted interval of slow wave and rapid eye movement sleep, as well as for one-minute samples during apnea and during slow wave and rapid eye movement sleep after resumption of respiration.
RESULTS: Compared to the placebo, sildenafil was associated with an increase in the normalized high-frequency (HF_{nu}) components and a decrease in the low/high-frequency components of the heart rate variability ratio (LF/HF) in slow wave sleep ($p<0.01$ for both). Differences in heart rate variability parameters between one-minute post-apnea and apnea samples (Δ= difference between resumption of respiration and apnea) were assessed. A trend toward a decreasing magnitude of ΔLF activity was observed during rapid eye movement sleep with sildenafil in comparison to placebo ($p=0.046$). Additionally, Δ LF/HF in SWS and rapid eye movement sleep was correlated with mean desaturation ($s_{R=}$ -0.72 and -0.51, respectively, $p= 0.01$ for both), and Δ HF_{nu} in rapid eye movement sleep was correlated with mean desaturation ($s_{R=}$ 0.66, $p= 0.02$) and the desaturation index ($s_{R=}$ 0.58, $p = 0.047$).
CONCLUSIONS: The decrease in arousal response to apnea/hypopnea events along with the increase in HF_{nu} components and decrease in LH/HF components of the heart rate variability ratio during slow wave sleep suggest that, in addition to worsening sleep apnea, sildenafil has potentially immediate cardiac effects in patients with severe obstructive sleep apnea.

KEYWORDS: Nitric oxide; Heart rate variability; Phosphodiesterase-5; Erectile dysfunction; Oxyhemoglobin.

INTRODUCTION

Obstructive sleep apnea (OSA) is associated with a number of conditions prevalent among middle-aged men[1-4] and is a major factor contributing to erectile dysfunction.[5] Concerns have arisen about the increasing percentage of men using selective phosphodiesterase-5 (PDE-5) inhibitor drugs to treat erectile dysfunction.[6] Our previous research showed that this concern is justified; sildenafil has a magnifying effect on respiratory events in patients with severe OSA.[7] Furthermore, OSA remains undiagnosed in 80% of patients.[8]

Our previous study found that an increase in the number and duration of obstructive respiratory events might be related to the nasal congestion that is frequently reported by sildenafil users.[9] These events could also be linked to a ventilation-perfusion mismatch[10] related to the nitric oxide (NO)-dependent vasodilatory effect of sildenafil in the absence of ventilation. Although we observed worsening apnea and oxyhemoglobin desaturation, our study found an interesting less-than-expected increase in arousal.[7]

The blunted arousability of OSA patients after sildenafil consumption may result from cerebral vasodilation due to increased cerebrovascular reactivity to hypercapnia.[11] However, the blunted arousability may also reflect an

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impaired autonomic nervous system response.

Research has shown an association between cardiac autonomic function measures and the severity of sleep-disordered breathing.[12] Heart rate variability (HRV) is not the most direct measure of autonomic activity but is widely used because of its non-invasiveness and ease of application.[13] In patients with OSA, HRV differs in apnea and post-apnea periods. The increased negative intrathoracic pressure (the Muller maneuver) during respiratory effort arises from parasympathetic predominance.[14] However, during apnea events, a progressive increase in sympathetic activity also occurs due to oxyhemoglobin desaturation.[15] The maximum sympathetic activity occurs at the end of the apnea event; from then on, sympathetic activity decreases until the recovery of normal respiration.[16] A bilateral relationship exists between arousal and sympathetic activity in OSA[17], and both are related to sleep fragmentation.

To the best of our knowledge, the influence of sildenafil on autonomic nervous modulation in patients with severe OSA has not yet been reported. The primary goal of the present study is to examine this relationship during slow wave sleep (SWS) and rapid eye movement (REM). A secondary objective is to evaluate sildenafil-induced modifications of HRV during apnea and after the resumption of respiration.

PATIENTS AND METHODS

Heart rate variability recordings were performed during polysomnography (PSG) for the thirteen subjects included in our previous study.[7] These recordings were evaluated and compared with the patients' PSG respiratory parameters.

Patients included in the study were between the ages of 40 and 65 years and had a body mass index (weight in kilograms/height in meters squared) of less than 30, an apnea-hypopnea index (AHI) of more than 30 events/hour of total sleep time (TST), and an oxygen desaturation (>4%) index of 10 or more/hour of TST, as evidenced by polysomnography performed less than six months earlier.

Exclusion criteria included daytime hypoxemia, concomitant significant debilitating illness, the use of nitrates or drugs that could influence sleep, current alcohol or drug abuse, and a current or previous habit of smoking more than 10 cigarettes a day. Further exclusion criteria included acute or chronic respiratory disease based on symptoms and respiratory function test results, systemic arterial hypertension, and evidence of previous or present cardiac disease based on symptoms, 12-lead electrocardiography, or Doppler echocardiography. Subjects who participated in trials with continuous positive airway pressure devices in the previous six months were also eliminated from the study.[7]

The study protocol was approved by the local Ethics Review Committee, and all participants signed written informed consent forms.

Study design

After consenting to participate in the study, all of the patients were evaluated for the presence of vascular or metabolic disease (i.e., arterial hypertension, diabetes, coronary artery disease, cerebrovascular disease, hypercholesterolemia, and diabetes mellitus) and smoking habits. To exclude patients with cardiac and pulmonary hypertension (defined as a pulmonary arterial pressure above 20 mmHg) and other respiratory diseases, electrocardiography, Doppler echocardiography, and respiratory function tests were performed.

Polysomnography recordings were carried out after a night of adaptation to the sleep laboratory. Each participant received two coded envelopes, one containing the drug and the other containing the placebo, and was asked to randomly select one of the envelopes and take the pill that was inside at bedtime. The pill in the remaining envelope was administered on the next recording night. The codes were opened at the end of the study.

Polysomnography

Bedtime was based on each patient's habits. At least seven hours of recording time were obtained. The following measurements were collected: an electroencephalogram (at positions C3-A2, C4-A1, and O1-A2 of the International 10-20 System), a bilateral electrooculogram, a submental electromyogram, and an electrocardiogram (modified V_2 lead).

Respiration was monitored as follows: airflow was measured with a nasal cannula/pressure transducer system (Pro-Tech Services Inc; Mukilteo, WA, USA) and a mouth thermocouple; chest and abdominal efforts were measured with uncalibrated, inductive, respiratory plethysmographic belts; arterial oxygen saturation (SaO_2) was measured with pulse oximetry (Ohmeda Hatfield, Herts., England), and body position movements were measured with a mercury gauge. Body position was determined by a sensor. Data were collected using a 16-channel computerized sleep system (Harmonie 5.2; Stellate Systems Inc., Montreal, Quebec) with a sampling rate of 512 Hz.

An experienced researcher, blinded to the medication condition of the participants, performed the sleep scoring of each patient's three PSGs; the scoring was conducted according to previously established parameters.[18] Total sleep time (TST) was defined as the time elapsed between the first

and last recorded epoch of sleep, excluding wakefulness. Arousals lasting more than 3 seconds were scored according to the criteria established by the American Sleep Disorders Association.[19]

According to the parameters established by the Taskforce of the American Academy of Sleep Medicine,[20] apnea was defined as a period of breathing cessation and hypopnea, as a 50% reduction in breathing, or as less than a 50% reduction in breathing associated with a 4% desaturation of oxyhemoglobin or arousal. The minimum duration of an event was ten seconds. The apnea hypopnea index was defined as the total number of apneas and hypopneas/hour of TST. Obstructive AHI was defined as the number of obstructive apneas plus hypopneas/hours of TST, mixed AHI was defined as mixed apneas plus hypopneas/hours of TST, and central AHI was defined as central apneas plus hypopneas/hours of TST. The percentage of TST spent in apnea/hypopnea events (%TST AH) was calculated to estimate the duration of respiratory events during sleep. The desaturation index (DI) corresponded to the number of arterial oxygen desaturations (with a drop greater than 4%) per hour of TST. The percentage of TST spent with less than 90% oxyhemoglobin saturation (Sat O_2<90%) was also measured.

Heart rate variability

Frequency domain analysis of HRV[21] was performed for the central five-minute sample of the longest interval of SWS and REM sleep that was free of stage shifts, artifacts or arousals.[22] Additional frequency domain analysis of HRV was carried out in one-minute samples during apnea and after the opening of the upper airway in SWS and REM. Samples during an apnea event were eligible only if they occurred during obstructive events and were followed by a post-apnea one-minute period that was free of further apnea or artifact events. The interval corresponding to the opening of the airway was excluded.[23] The spectra of the selected samples were adjusted for respiration using the Welch method with 128-sample Hanning windowing.[24]

This study focused on the low frequency band (LF: 0.04 to 0.15 Hz), which is influenced by sympathetic activity, and on the high frequency component (HF: 0.15 to 0.40 Hz), which is under vagal control, is synchronous with respiratory frequency, and represents vagal heart activity.[13] The LF/HF ratio reflects sympathovagal modulation.[25] Normalized ($_{nu}$) values and spectral analysis were used to quantify changes in the components of HRV. This method is more sensitive to fluctuations in cardiac autonomic influence than time domain indexes of HRV because the spectral measures are normalized ($_{nu}$) in relation to total power (e.g., LF_{nu} = LF/total power and HF_{nu}= HF/total power).[26] The power density in the very low frequency range (VLF: 0.0033 to 0.04 Hz) was not analyzed because short-term HRV analysis cannot detect this component. Because the total frequency (TF) includes the sum of very low, low, and high frequency power, TF values were also not included in this analysis.[21]

Statistical analysis

A Shapiro-Wilk test was used to examine the normality of the distribution, and values were expressed as mean ± standard error (SE) (Table 1). The differences among groups were analyzed using a dependent sample t-test or Wilcoxon test. Differences in HRV parameters between one-minute post-apnea and apnea periods (Δ= difference between post-apnea and apnea) were assessed. In addition, a Spearman test was used to determine correlations between ΔHRV parameters and the following respiratory variables: mean desaturation of oxyhemoglobin, AHI, DI and respiratory arousal index. Statistical evaluations were conducted with Statistica (version 6.0) for Windows software (Statsoft, Inc., Tulsa, OK, 2004). Significance was established at p<0. 05.

RESULTS

Table 1 shows the effects of sildenafil and the placebo on HRV and respiratory parameters during SWS and REM sleep. As described in our previous study,[7] we observed an increase in mean desaturation (p= 0.02), in the apnea hypopnea index (p= 0.0006), and in the desaturation index (p=0.002) as well as a decrease in mean saturation (p=0.01) after sildenafil treatment in comparison to the placebo. An increase in HF (p=0.0001) and a decrease in LF/HF in SWS sleep (p=0.002) were also observed after sildenafil treatment. The respiratory arousal index did not significantly differ in relation to the treatments, but a decrease in SWS, in terms of both minutes and percentage of TST, was observed after sildenafil.

A decreased magnitude of the difference (Δ) in the LF component of HRV was observed in REM sleep after sildenafil treatment in comparison to the placebo (p=0.046). A clear trend toward a decrease in Δ LF/HF was also observed in REM sleep after sildenafil (Figure 1).

Examinations of the correlations between HRV and respiratory parameters showed that Δ LF/HF in SWS and REM sleep was correlated with mean desaturation (s_R = -0.72 and -0.51, respectively, p = 0.01 for both), whereas Δ HF in REM sleep was correlated with mean desaturation (s_R=0.66, p = 0.02) and the desaturation index (s_R = 0.58, p = 0.047).

Table 1 - Analysis of heart rate variability in slow wave sleep and REM sleep. Respiratory sleep parameters after placebo and sildenafil.

	Sleep parameter	Placebo	Sildenafil	p
SWS	LF	0.74 (0.04) §	0.72 (0.03)	0.81
	HF	0.12 (0.02)	0.28 (0.03)	0.0001
	LF/HF	10.14 (2.29) §	3.57 (0.62)	0.002
	SWS min	77.0 (7.3)	53.3 (4.4)	0.001
	%TST	17.1 (1.4)	12.5 (0.9)	0.001
	Respiratory arousal index	0.5 (0.2)	0.7 (0.2) §	0.16
REM	LF	0.75 (0.04)	0.74 (0.04)	0.89
	HF	0.25 (0.04)	0.26 (0.04)	0.89
	LF/HF	5.33 (1.05)	5.09 (1.29) §	0.92
	REM min	90.1 (8.4)	91.1 (5.8)	0.89
	%TST	20.3 (1.5)	21.3 (1.1)	0.38
	Respiratory arousal index	17.3 (1.9)	18.2 (1.9) §	0.22
Respiratory data	Saturation (mean)	93.8 (0.4)	92.1 (0.5)	0.01
	Desaturation (mean)	8 (1.3) §	9.9 (1.3) §	0.02
	Apnea hypopnea index	32.3 (3.1)	48.0 (5.7) §	0.0006
	Desaturation index	18.5 (2.5)	30.3 (4.0) §	0.002

Mean (standard error). § Data with nonparametric distribution. Heart rate variability was analyzed in the central five-minute sample of the longest interval of slow wave sleep (SWS) and REM sleep that was free of stage shifts, artifacts or arousals.

DISCUSSION

This double-blind, cross-over, placebo-controlled study builds on data from our previous research on the respiratory effects of sildenafil in 13 patients with severe OSA.[7] The present study examined the impact of sildenafil on HRV during SWS and REM sleep as well as differences in HRV between apnea and respiration resumption. We used short-term frequency domain analysis throughout the sleeping period and paid special attention to high-frequency and low-frequency ranges.

Our previous study found that sildenafil was correlated with impaired respiratory parameters during sleep (e.g., mean values of saturation, desaturation, apnea hypopnea index and desaturation index) that were not followed by an increase in the respiratory arousal index.[7] This study found that sildenafil was correlated with an increase in HF_{nu} and a decrease in the LF/HF components of HRV during SWS but not during REM sleep. We examined the mean difference in HRV parameters (Δ) between post-apnea and apnea events and identified a trend toward a reduced modification of LF_{nu} and of the LF/HF components of HRV during REM sleep with sildenafil in comparison to the placebo.

These findings suggest that, in patients with severe OSA, a single dose of sildenafil (50 mg) before sleep affects sleep HRV, with predominance of parasympathetic upon sympathetic tone. This was evident in SWS, but not in REM, in which greater sympathetic tone typically occurs.[12] Furthermore, the trend toward less modification of LF_{nu} and of the LF/HF components of HRV during REM sleep with sildenafil suggests that either the increased LF overshadows the HF increment in this sleep stage or that a ceiling effect occurs in the sympathetic tone that is related to the severity of respiratory events.[12, 15, 16]

In the evaluation of post-apnea and apnea modification in HRV, the first open breath was excluded because of the typical surge of sympathetic activity triggered by hypoxia or by the activation of carotid and/or aortic chemoreceptors.[27] As found in previous studies (see Younes[27] for reviews), the amplitude of the modification of HRV parameters had relatively little influence on the number of apnea events in these patients.

This research is clinically important because abnormal autonomic control is a key factor in the causal link between OSA and cardiovascular disease.[14,16] Apnea is associated with increased circulating norepinephrine and with the loss of vagal tone, a combination that may underlie life-threatening arrhythmias.[28] Long-term complications, i.e., hypertension, myocardial infarction, and stroke, may result from repeated temporary loss of NO in tissues. This loss of NO is produced by a lack of oxygen, one of the two essential substrates of NO.[10] Manipulation of the NO/cyclic GMP system may therefore be a therapeutic option in this circumstance. Our finding that the HF component of HRV increases in severe OSA patients after the use of sildenafil shows that caution must be exercised when changes in HRV spectral indices

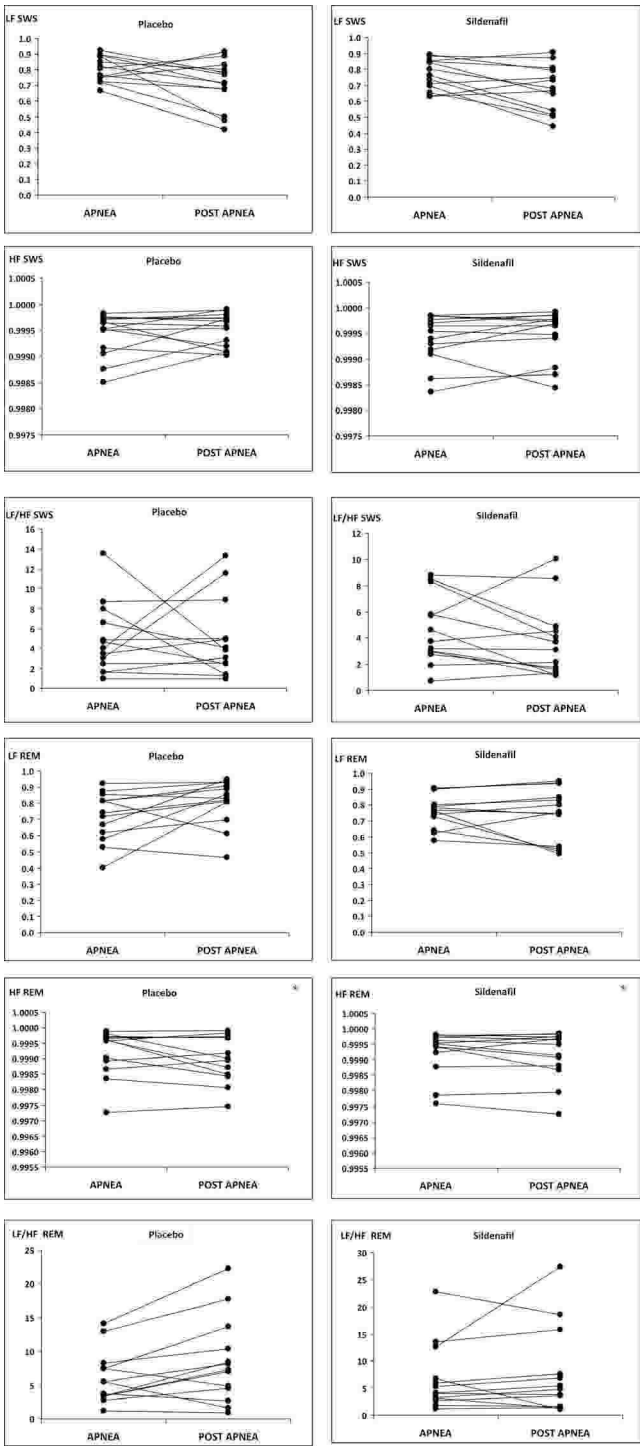

Figure 1 - Modification of HRV parameters in post apnea and apnea events.

are interpreted to represent changes in vagal or sympathetic activities.[29] Sometimes, estimates of HF do reflect heart rate variability;[30] instead, they reflect both non-respiratory sinus arrhythmia and respiratory instability. The emergence of hyperpneic breaths following the termination of apnea can increase estimates of HF, leading to the erroneous interpretation of increased vagal tone during these episodes.[31]

The influence of sleep stage on respiration and HRV has been reviewed in detail.[12] The shift from wakefulness to non-REM sleep is accompanied by a progressive increase in parasympathetic modulation and a decrease in sympathetic modulation of the heart rate. On the other hand, REM sleep, which typically occurs at 90-minute intervals, is associated with significantly greater sympathetic modulation than non-REM sleep. In addition, ventilation and breathing patterns vary according to sleep stage; non-uniform ventilatory patterns can substantially influence LF and HF, particularly in sleep-disordered breathing conditions. High-frequency power is closely associated with the respiratory modulation of heart rate, commonly known as respiratory sinus arrhythmia. Both tidal volume and breathing frequency compromise the reliability of the HF component of HRV as a measure of parasympathetic activity.[31]

Clinically, OSA is a REM-sleep related disease; obstructive events occur more frequently, for a longer duration of time, and with more desaturation of oxyhemoglobin during REM sleep than non-REM sleep.[32] Variable surges in sympathetic activity are associated with a range of arousal events. Arousals can vary from increases in muscular and respiratory activity to sleep stage shifts and full awakening.[33] Changes at the cortical level may not be visible in SWS because the magnitude of the autonomic activation is lower than in light and REM sleep. Previous studies reported that around 30% of apneas/hypopneas, particularly in NREM sleep, are not terminated by visible cortical arousals.[34] The present study provides evidence that, after administration of sildenafil, either hypoxemia or increased upper airway resistance due to nasal congestion may lead to a lack of arousals during SWS as a response to apneas and hypopneas. The same was not observed in REM sleep due to eased sympatho-vagal balance and arousability.[35] Exacerbation of obstruction and an increase in inspiratory effort make upper airway pressure even more negative. An ineffective pharyngeal dilator reflex may also impair arousal and lead to central apnea events in OSA.[28]

It is also possible that the profound change in respiratory pattern observed in the cases of severe OSA in this study may have had a direct influence on the excitatory drive of peripheral and central chemoreceptors after sildenafil administration. In addition to a direct effect on chemoreceptor afferent input, an indirect action at the level of central respiratory drive may profoundly influence vagal efferent activity.[36] The predominant cholinergic control of airway smooth muscle at the level of the neuro-effector junction has recently been reviewed in detail by Jordan[36] and the anatomical organization of airway pathways in the brainstem has been reviewed by Mazzone and Canning.[37]The data presented in this study also raise important questions

about the role of NO in the responsiveness of the cerebral vasculature to changes in blood gas tension as well as to sildenafil. NO plays a direct role in obstructive apnea events; during these events, there is an intermittent failure to transport nasal NO to the lungs with each breath and a decrease in NO synthesis due to the lack of oxygen.[38] In this context, sildenafil, a PDE-5 inhibitor, potentially impairs NO-dependent protective mechanisms of adaptation to intermittent hypoxia. For example, it may affect the compensatory mechanism that matches perfusion to ventilation and alter efferent pathways that control the activity of pharyngeal dilators and thoracic musculature.[10, 39]

Furthermore, neuronal and endothelial NO exerts an inhibitory nitrergic influence on both inotropic and chronotropic cardiac responses to adrenergic stimulation.[40] NO acts to increase acetylcholine release in cholinergic neurons. Conversely, NO generated in the sympathetic ganglia reduces the release of noradrenaline.[41]

The results of our study are also supported by the findings of Chowdhary et al.,[42] which demonstrated that enhancement of baroreflex gain by NO may underlie the modulation of cardiac vagal control. An increase in HF, but not in LF, was observed with NO-synthetase drug inhibitors and exogenous NO donors.

In addition to the reported involvement of NO in sleep termination, other studies have reported that NO[43] has a somnogenic effect in OSA patients (see Gautier-Sauvigné[44] for a review of this literature). These studies support our finding that the use of sildenafil is associated with blunted arousal response to respiratory events during sleep.

Our data on the modification of HRV components between post-apnea and apnea events are heterogeneous; this may highlight the individual pattern of collapsibility of the upper airway and of ventilatory instability. The degree of airway collapsibility and the necessary effort required to open it are inversely proportional to and dependent on the degree of arousal.[45]

Several methodological considerations must be acknowledged. These include the application of HRV analysis to non-stationary conditions such as sleep in OSA patients. To overcome this difficulty, we used spectral analysis of central 5-minute samples of the longest interval of SWS and REM sleep that was free of stage shifts, artifacts or arousals.[23] However, because AHI was high, particularly after the use of sildenafil, it was not possible to obtain five-minute samples of SWS or REM sleep that were free of apnea or hypopnea events; adjustments for respiration were performed in the spectra of the selected samples.[24]

This study has several limitations. First, we estimated changes in heart frequency using HRV rather than baroreceptor reactivity. However, it was not feasible to perform recordings of baroreflex or vagal efferent activity which are supposedly impaired during sleep in severe OSA patients. Second, overnight measures of CO_2 and the number of central sleep apnea events would provide additional information regarding chemoreceptor influence on HRV parameters. Third, sildenafil is designed to influence penile erection for 2 to 5 hours; this is less time than a full night of sleep. However, little is known about the duration of the other effects of this drug. Further studies of long-term PDE-5 inhibitors such as vardenafil and of daily sildenafil doses are needed to improve our understanding of this group of drugs. Finally, the small sample size of this study raises questions about the applicability of results. The data from the 13 patients, however, show a consistently low ratio of arousals and apnea/hypopnea events during SWS and REM sleep, as well as increased desaturation and lower saturation after the use of sildenafil. Additionally, because all eligible recorded apneas/hypopneas were similarly analyzed, the large amount of data may be representative of the characteristics of the respiratory events.

The lack of increase in the arousal index during SWS may suggest a link between the worsening of OSA and HRV abnormalities after sildenafil. These findings provide evidence of a potential risk of sildenafil use by OSA patients with cardiovascular disease. Our data support Stein et al.'s suggestion that increases in HF estimates do not always reflect better heart rate variability.[30]

In conclusion, the decrease in arousal response to apnea/hypopnea events along with the increase in HF and decrease in LH/HF components of heart rate variability during SWS suggest that, in addition to worsening sleep apnea, sildenafil has potentially immediate cardiac effects in patients with severe obstructive sleep apnea.

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