Inspiratory-resistive loading increases the ventilatory response to arousal but does not reduce genioglossus muscle activity on the return to sleep

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Submitted 16 May 2012; accepted in final form 15 July 2012

Cori JM, Nicholas CL, Baptista S, Huynh I, Rochford PD, O’Donoghue FJ, Trinder JA, Jordan AS. Inspiratory-resistive loading increases the ventilatory response to arousal but does not reduce genioglossus muscle activity on the return to sleep. J Appl Physiol 113: 909–916, 2012. First published July 19, 2012; doi:10.1152/japplphysiol.00608.2012. —Arousals from sleep are thought to predispose to obstructive sleep apnea by causing hyperventilation and hypocapnia, which reduce airway dilator muscle activity on the return to sleep. However, prior studies of auditory arousals have not resulted in reduced genioglossus muscle activity [GG-electromyogram (EMG)], potentially because airway resistance prior to arousal was low, leading to a small ventilatory response to arousal and minimal hypocapnia. Thus we aimed to increase the ventilatory response to arousal by resistive loading prior to auditory arousal and determine whether reduced GG-EMG occurred on the return to sleep. Eighteen healthy young men and women were recruited. Subjects were instrumented with a nasal mask with a pneumotachograph, an epiglottic pressure catheter, and intramuscular GG-EMG electrodes. Mask CO2 levels were monitored. Three- to 15-s arousals from sleep were induced with auditory tones after resting breathing (No-Load) or inspiratory-resistive loading (Load; average 8.4 cmH2O·l−1·s−1). Peak minute ventilation following arousal was greater after Load than No-Load (mean ± SE: 8.0 ± 0.6 vs. 7.4 ± 0.6 l/min, respectively). However, the nadir end tidal partial pressure of CO2 did not differ between Load conditions (43.1 ± 0.6 and 42.8 ± 0.5 mmHg, respectively), and no period of reduced GG activity occurred following the return to sleep (GG-EMG baseline, minimum after Load and No-Load = 2.9 ± 1.2%, 3.1 ± 1.3%, and 3.0 ± 1.3% max, respectively). These findings indicate that the hyperventilation, which occurs following tone-induced arousal, is appropriate for the prevailing level of respiratory drive, because loading did not induce marked hypocapnia or lower GG muscle activity on the return to sleep. Whether similar findings occur following obstructive events in patients remains to be determined.

Obstructive sleep apnea; airway dilator muscles; ventilation; hypocapnia

The pathogenesis of obstructive sleep apnea (OSA) is multifactorial and incompletely understood (7). Whereas impaired upper-airway anatomy and sleep-related changes in dilator muscle function appear critical, the role of other factors, such as arousal from sleep, is less clear. Arousals are associated with increased dilator muscle activity, as well as hyperventilation and a lowering of the end tidal partial pressure of CO2 (PETCO2) (12, 17). The activity of upper-airway dilator muscles varies with respiratory stimuli (19, 20), and therefore, the relative hypocapnia that develops during arousal has been suggested to cause dilator muscle hypotonia on the return to sleep, predisposing the individual to further obstructive respiratory events (4, 6, 16, 26).

During stable sleep, there is good evidence that hypocapnia reduces dilator muscle activity and increases upper-airway resistance (RUA), particularly in individuals with high airway resistance at baseline (2, 9, 22, 24). However, that the reduced PETCO2, which occurs following arousal from sleep, causes a similar predisposition to obstructive events on the return to sleep is not well supported by current literature. Specifically, three studies have investigated dilator muscle responses following tone-induced arousal from sleep in healthy controls or OSA patients on continuous positive airway pressure (CPAP) and failed to observe low muscle activity at any time during arousal or on the return to sleep (5, 12, 25). Two studies further measured RUA following the return to sleep after brief tone-induced arousals in patients with OSA who were studied on suboptimal or therapeutic CPAP (14, 18). Both studies reported that resistance was reduced, not increased, as would be expected if the airway were more collapsible (14, 18). In addition, very few respiratory events occurred following tone-induced arousals, even when the patients were on subtherapeutic CPAP (14).

Possible explanations for the prior studies failing to demonstrate low dilator muscle activity on the return to sleep after arousal are that: 1) the magnitude of hyperventilation (and resultant hypocapnia) with tone-induced arousal may be smaller than the hyperventilation/hypocapnia that occurs at the end of naturally occurring respiratory events in OSA; 2) the magnitude of hyperventilation at arousal may be appropriate for the level of respiratory drive present prior to arousal, and therefore, hypocapnia is not induced; or 3) as we have proposed previously (13), there may be independent activation of dilator muscles at arousal from sleep, which results in persistently high dilator muscle activity on the return to sleep, independent of other inhibitory stimuli such as hypocapnia. We aimed to investigate these possibilities by increasing the ventilatory response to arousal in healthy controls by resistive loading prior to auditory arousal and assessing the dilator muscle response. We hypothesised that inspiratory-resistive loading (Load) would increase the hyperventilation at arousal from sleep, leading to greater hypocapnia, but that the activity of the genioglossus (GG) muscle on the return to sleep would be unchanged.

Methods

Subjects. Healthy young men and women were recruited by advertisements placed around the University of Melbourne. Subjects were nonsmokers with no sleep or other medical problems,
who had not traveled through time zones in the month prior to testing and had regular sleep patterns. No subject took medication, and women were tested in the follicular menstrual phase. All subjects gave written, informed consent, and the study was approved by the University of Melbourne Institutional Ethical Review Board.

**Equipment and techniques.** Subjects were instrumented with two EEGs (C3 and F3 referenced to A2), left and right electrooculogram (EOG), and mentalis (chin) electromyogram (EMG) for sleep staging and arousal scoring. The mentalis muscle was used, because submental EMG is often very close to the location for percutaneous intramuscular GG-EMG electrode insertion. Fine-wire intramuscular electrodes (p/n 000-318-30; Motion Lab Systems, Baton Rouge, LA) were used to record the GG-EMG. Two electrodes were placed percutaneously 3–4 mm from the midline to a depth of ~20 mm for a bipolar recording. Surface diaphragm EMG recordings were made with electrodes placed in the seventh to ninth intercostal spaces, adjacent to the costal margin. All EMGs were amplified and band-pass filtered from 30 to 1,000 Hz (model PS11, Grass TeleFactor; Grass Technologies, West Warwick, RI) before being scaled between electrical zero and each subject’s voluntary maximal activity (see Protocol). A pressure transducer-tipped catheter (model MCP-500; Millar, Houston, TX) was inserted through one anesthetized nostril (2% Lidocaine HCl gel) until the catheter tip was located at the epiglottis (10–20 mm below the tongue base). A leak-proof nasal mask (modified Profile Lite; Philips Respironics, Murrysville, PA) was then placed over the subject’s nose and connected to a heated pneumotachograph (model 3700; Hans Rudolph, Shawnee, KS), attached to a two-way valve (model 2600; Hans Rudolph) to separate inspiratory and expiratory flow. The inspiratory side of the valve was attached to tubing, which passed through the bedroom wall to a three-way tap connected to variable linear-resistive loads (5–20 cmH2O·1·s−1; model 7100 R5; Hans Rudolph) via one port or open to room air through the other. The partial pressure of CO2 was sampled continuously from the mask and Hans Rudolph) via one port or open to room air through the other. The PETCO2 values were deleted for any breaths without a plateau visually inspected on all breaths to ensure that a plateau occurred, and PETCO2 values were deleted for any breaths without a plateau (usually associated with small expiratory volumes). Similarly, the GG muscle tracing was visually inspected for all breaths, and artefacts related to swallows or electrical interference were removed.

The following types of events were averaged within each subject: 3- to 15-s tone-induced arousals following No-Load (No-Load + Arousal); 3- to 15-s tone-induced arousals following Load (Load + Arousal); 0 dB control “tones” following No-Load (No-Load + No-Tone); and 0 dB control tones following Load (Load + No-Tone; example shown in Fig. 1). Tones that did not result in 3- to 15-s arousals were also examined, as these were often associated with signs of subcortical arousals, such as <3 s of alpha activity in the EEG (No-Load + No-Arousal and Load + No-Arousal). Respiratory and muscle data for tone trials resulting in arousal were compared with a 2 (Load and No-Load) × 10 (10 breaths following Arousal) repeated-measures ANOVA. Separate 2 × 10 ANOVAs were performed for No-Tone and No-Arousal conditions. Student’s t-tests were used to compare respiratory and muscle data between wake and sleep as well as Load and No-Load prior to auditory tone presentation. Mean ± SE are reported, and P < 0.05 was considered statistically significant.

**RESULTS**

Eighteen subjects were recruited to the study. Five subjects slept poorly in the laboratory and either had no sleep or did not have tones that induced 3- to 15-s arousals in both Load and No-Load conditions. Another subject slept extremely soundly, and brief arousals could not be induced even with the loudest tone (100 dB). A further two subjects were found to have repeated hypnopenias while in the supine position with all monitoring equipment attached and were excluded. Thus adequate data were obtained in 10 subjects, who were aged 20.7 ± 0.5 yr and had a body mass index of 22.0 ± 1.1 kg/m2. Two of these subjects could not sleep while supine and so were studied in the lateral position.

Subjects had a normal average sleep onset latency of 13.3 ± 7 min but as a result of the experimental equipment and
protocol, had relatively low sleep efficiency (71.9 ± 6.7%) and high arousal index (29.5 ± 2.8 arousals/h of sleep). The total sleep time was 216.8 ± 27.4 min, which was comprised of a relatively high percentage of N1 and N2 sleep (17.9 ± 4.1% and 62.2 ± 3.6%, respectively) with relatively little N3 or REM (12.5 ± 2.1% and 7.4 ± 2.3%, respectively). The changes in respiratory and muscle activity from wakefulness to stable sleep at the beginning of the night are shown in Table 1. As expected, VT, F_i, and PETCO_2 rose. However, airway resistance did not rise during sleep in these young, healthy subjects, and GG muscle activity during sleep was not statistically different from the waking level.

On average, 33.3 ± 6.5 tones and 5.1 ± 0.9 control tones (0 dB) were played/subject. These resulted in an average/subject of 4.3 ± 1.0 Load + Arousal, 5.5 ± 1.3 No-Load + Arousal, 2.7 ± 0.4 Load + No-Tone, and 3.0 ± 0.5 No-Load + No-Tone trials. Many of the remaining tones were followed by subcortical arousals characterized by EMG or cardiorespiratory activation with no or subcriterion changes in the EEG, such as <3 s of increased frequency EEG. The average volume of tone that elicited arousal was 60.6 ± 4.5 dB and did not differ between load conditions. The duration of AASM arousal did not differ between loading conditions (7.1 ± 0.7 s after Load and 8.5 ± 0.9 s following No-Load).

The effects of Load prior to auditory tone presentation in the trials that resulted in AASM arousal, are presented in Table 2 (the average of the last two breaths before the tone is presented). The average resistive load used was 8.4 ± 0.9 cmH2O·l−1·s−1. As expected, load reduced V_t and V_T, augmented mask, and P_EPI swings (more negative) and increased the duty cycle (T_I/T_TOT). However, PETCO_2, RUA, diaphragm, and GG muscle activities did not change significantly with loading.

The physiologic changes following arousal from sleep in the Load and No-Load conditions are shown in Fig. 2. Significant ANOVA main effects for load condition were observed for V_t and V_T. Both variables were higher in the Load than No-Load condition overall across all breaths. Peak GG activity showed a trend toward a significant main effect for load condition (P = 0.055), and no other variables showed a significant main effect for load. Significant main ANOVA effects for breath were found for V_t, V_T, T_I/T_TOT (not shown), PETCO_2, RUA, and that elicited arousal was 60.6 ± 4.5 dB and did not differ between load conditions. The duration of AASM arousal did not differ between loading conditions (7.1 ± 0.7 s after Load and 8.5 ± 0.9 s following No-Load).

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both Peak and Tonic GG and diaphragm activity. Only one significant interaction effect was observed, and that was for GG Peak, indicating that GG activity was initially higher in the Load compared with No-Load condition but that this difference did not persist for all 10 breaths. Given that the time at which participants returned to sleep varied between arousals, an additional analysis was conducted that aligned the data by the breath on which the subject returned to sleep. Importantly, the average number of breaths before the subjects returned to sleep did not differ between load conditions. Furthermore, statistical analysis on the first 10 breaths after the return to sleep showed near-identical results to those shown in Fig. 2, suggesting that the duration of arousal did not influence the findings.

When 0-dB control tones were played, there also tended to be a small, nonsignificant increase in ventilation following Load compared with No-Load (Fig. 3). Therefore, to account for the physiological effects of load removal alone, the difference between Arousal and No-Tone (control) trials for both Load and No-Load was calculated (Fig. 4). Although main ANOVA effects for breath were found for \( V_I \), \( V_T \), \( T_I/T_{TOT} \),
PETCO₂, and GG Peak, No-Load or Load-by-Breath interactions were observed.

Given the large number of tones, which did not result in arousal (either no visible EEG response or small EEG changes that did not reach the AASM criteria), we compared the physiologic response to tones that ended with and without AASM arousal. This analysis was performed in both the Load and No-Load conditions separately. However, the results were very similar, and therefore, only the No-Load condition is presented in detail. The volume of tone played did not differ between tones that elicited AASM arousal and those that did not (58.7 ± 5.2 and 65.8 ± 3.4 dB, respectively; P = 0.5). However, the sleep stage was more often N3 than N2 in trials that did not induce an arousal (ratio N2:N3: Arousals 1:0.13 vs. No-Arousals 1:0.74). The physiologic changes following tones that did not induce an arousal were identical to those that resulted in an AASM arousal (V̇ₐ, PETCO₂, Peak GG, and Rₚₐ on the return to sleep were not different between load conditions). The horizontal dotted line represents the average ventilation during the 5 prearousal breaths in the No-Load condition.

**DISCUSSION**

The major finding of this study is that Load increased the ventilatory response to arousal from sleep but did not induce more hypocapnia or reduce the GG muscle activity on the return to sleep. Importantly, when the physiologic responses following control tones (0 dB) were subtracted from noncontrol tones, there was no longer any difference between load conditions (Fig. 4). This suggests that load removal and arousal have separate and additive physiologic effects and do not synergistically act to raise ventilation following arousal. It is therefore not surprising that the changes in PETCO₂ following arousal were not different between load conditions, because subjects were mildly hypercapnic during loading (not significantly different but ~0.6 mmHg higher than in the No-Load condition), and therefore, the enhanced hyperventilation in the loading condition resulted in very similar CO₂ levels on the return to sleep. OSA patients have a large “load” present prior to respiratory event termination and also have marked hyperventilation after arousing from sleep at respiratory event termination (26). It has been proposed that the marked hyperventilation seen in OSA may be excessive and lead to hypocapnia. However, we are unaware of any paper showing hypocapnia following respiratory event-induced arousal from sleep in naturally occurring OSA, potentially because CO₂ levels are very difficult to measure accurately during sleep in OSA (23). It is possible that many OSA patients, such as the healthy controls studied here, have appropriate hyperventilation that restores blood gases to baseline levels rather than inducing marked hypocapnia as suggested previously. Clearly further studies are required to investigate arousal responses in OSA before conclusions can be drawn, particularly given the relatively small loads that were tolerated in the current study, as well as the study of healthy subjects with very good upper-airway function. However, some additional support for the concept that the large hyperventilation seen in OSA is appropriate for the level of hypocapnia comes from our prior study of tone-induced arousals in OSA patients while on full or partially therapeutic CPAP (14). In that study, the magnitude of hyperventilation at arousal was enhanced while on subtherapeutic CPAP, and although the percent reduction in CO₂ was greater after arousals on subtherapeutic CPAP, the absolute levels of CO₂ on the return to sleep were not different between CPAP conditions (42.5 ± 0.9 mmHg while on low CPAP vs. 42.1 ± 1.0 mmHg on high CPAP; P = 0.17) (14). Thus it is possible that the large hyperventilation observed with arousal at the end of respiratory events in untreated OSA may also be appropriate to the prevailing level of respiratory drive and may not induce hypocapnia, at least in some patients.

Given that loading did not result in lower postarousal CO₂ levels than were observed in the No-Load condition following tone-induced arousals, it is not surprising that a greater reduction in GG muscle activity was not observed on the return to sleep. On the contrary, GG muscle activity was increased following arousal in both load conditions with a trend to be more elevated in the loaded compared with No-Load condition. That GG activity is not decreased following the return to sleep after arousal is consistent with three prior studies that have assessed dilator muscle activity following tone-induced arousals (5, 12, 25). Rₚₐ was not increased on the return to sleep in the current and prior (12, 14, 17, 18) studies, indicating that the upper airway is not more prone to collapse following return to sleep after arousal. These studies all used auditory tones to induce arousal, and as highlighted in the introduction, it is possible that the physiologic responses to respiratory event-induced arousal differ and that reduced dilator muscle activity occurs following respiratory event-induced arousals. However, we have recently reported increased GG muscle activity on the return to sleep following respiratory event-related arousals in OSA patients on reduced CPAP (13). Furthermore, we have demonstrated previously that GG muscle activity gradually increases across naturally occurring respiratory events in untreated OSA patients (15). Therefore, the lack of reduced GG muscle activity following the return to sleep after arousal may be a generalized finding in both OSA patients and healthy individuals.

Many tones in the current study did not induce arousals according to the AASM criteria (10). However, cardiorespiratory activation or subtle changes in the EEG were often discernable. We have reported recently that respiratory events in OSA patients, which end without a cortical arousal, were accompanied by near-identical physiologic changes to events ending with cortical arousal when the severity and duration of the respiratory event were matched (13). For this reason, we also compared the physiologic changes following auditory.
tones that did and did not induce AASM-defined arousals in the current data set. Consistent with the prior study in OSA patients, we found that the physiologic changes following auditory tones did not differ between tones ending with or without an AASM arousal (Fig. 5). Interestingly, the volume of the tone used did not differ between trials that did or did not result in arousal, but the trials ending without arousal occurred more commonly in slow-wave sleep. These results could be explained by the presence of a threshold for cardiorespiratory activation, which is lower than the threshold for EEG-defined arousal (drawn schematically in Fig. 6) and when reached, results in a stereotyped cardiorespiratory activation unrelated to the stimulus intensity. In this way, the tone volume may have exceeded the threshold for cardiorespiratory activation in both stage 2 and slow-wave sleep, but when the tone was played in slow-wave sleep [where the threshold for both events would be higher (3)], the threshold for EEG arousal was not reached as often, whereas the lower threshold for cardiorespiratory activation was still reached. Although other explanations of our findings are possible, we believe further research into the possibility of a cardiorespiratory activation threshold, which is slightly lower than the threshold that elicits AASM-defined EEG arousal, is warranted.

Despite its strengths, our study has some limitations. First, although the increase in ventilatory response to arousal following loading was statistically significant, the magnitude of additional hyperventilation was small. Khoo et al. (17) proposed that the magnitude of ventilatory response following arousal was predominantly related to changes in $R_{UA}$, as in his study, respiratory drive ($P_{0.1}$) was only increased for two breaths following arousal, whereas resistance was reduced for seven breaths and was correlated with the time course of increased ventilation. External Load minimally increased our subjects’ internal resistance in this study, and the load was removed immediately following presentation of the tone, potentially explaining the small increase in the magnitude of the ventilation response. Higher resistive loads may have resulted in greater resistance changes and more marked hyperventila-
tion, but this manipulation could not be used, as the healthy young subjects that we studied often awoke with higher resistances. Studying subjects who snore, are older, or have higher resistance asleep may be of interest in future studies. The second and related issue pertains to the small and nonsignificant changes in PETCO2 with loading. A step reduction in alveolar ventilation will result in an equivalent increase in PETCO2 in the steady state. Thus the small (16%) reduction in ventilation observed in the current study would have resulted in a 16% increase in PETCO2 if the reduced ventilation were maintained, resulting in a final PETCO2 of 50.8 mmHg. However, to reach steady state, the reduction in ventilation would have had to be maintained for many minutes (8, 11), whereas reduced ventilation only occurred for eight breaths (or approximately one-half of a minute—the typical duration of an apnea event), explaining why PETCO2 was altered minimally. Third, we only assessed 3- to 15-s (AASM-defined) arousals from sleep, and the physiologic changes may differ for arousals with duration outside of this range. However, given that we scored arousals according to the current clinical scoring criteria, we believe that the 3- to 15-s arousals are the most clinically relevant. Fourth, despite 18 subjects being studied, only 10 subjects had tone-induced arousals in both load conditions. Furthermore, there was some variability in responses among subjects. Thus it is possible that some of our comparisons were statistically underpowered, and the nonsignificant findings were a result of type 2 error. However, as can be seen in the figures, the nonsignificant differences appear near identical between load conditions, making it unlikely that any physiologically important differences were missed. Furthermore, if anything, PETCO2 was higher following Load than No-Load, and GG activity never fell below baseline in either condition. Therefore, even if a large number of subjects were studied, and these differences became statistically significant, the hypothesis that loading would result in greater hypocapnia and a period of dilator muscle hypotonia would still not be supported. Finally, two participants could not sleep in the supine position and so were studied laterally. Electrical vestibular stimulation has been used to alter GG activity in the cat (1), and we have previously shown differences in ventilatory response to arousal.

**Fig. 5.** Physiologic changes after tones that did and did not induce AASM arousal. Vl, Rl, PETCO2, and GG Peak for 10 breaths following auditory tones that did (No-Load + Arousal) or did not (No-Load + No Arousal) result in AASM arousal following No-Load. No significant ANOVA main or interaction effects were observed for arousal presence or absence. The horizontal dotted lines represent the average of the 5 prearousal breaths of each variable in both No-Load conditions. #Main ANOVA effect for breath.

**Fig. 6.** A model to explain the observation that the physiologic responses to tones ending without arousal were not different than those ending with AASM Arousal. It is possible that there is a threshold for cardiorespiratory activation in response to an arousing stimulus that is lower than the arousal threshold, but that varies with sleep stage in the same way that the arousal threshold does. During stage 2 sleep, the example 60-dB tone results in both thresholds being reached, and therefore, the subject has both an EEG arousal and activation of the cardiorespiratory system. However, the same tone played in slow-wave sleep (SWS) may no longer result in EEG arousal, as the arousal threshold has increased but is sufficient to cause cardiorespiratory activation. This would provide 1 explanation of why the physiologic responses to tones that did not result in AASM-defined arousal were not different than those tones that did induce arousal.