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**Methods:**

**Pre-planned secondary analysis:**

This study is a pre-planned secondary analysis of previously published experiments. The detailed data analysis plan was created after publication of the main results, prior to conducting any data analysis.

The primary aim of the original experiment was to investigate the effects of anesthetics on airway patency, upper airway muscle control, and swallowing. A manuscript focusing on the coordination between breathing and swallowing was published in Anesthesiology in 2014 (D’Angelo et al.; reference 15 in the original manuscript). A manuscript focusing on airway patency and upper airway muscle control was published in Anesthesiology in 2016 (Simons et al.; reference 16 in the original manuscript).

Once we had established the dose-dependent, impairing effects of anesthetics on upper airway muscle function, we aimed at investigating the hypothesis that CO2 insufflation can mitigate the detrimental effects of anesthetics on airway patency.

**Subject dropout:**

Eighteen volunteers were originally recruited. Several subjects called prior to the study date and elected not to participate, one did not show up, and one was not appropriately NPO. Of the remaining 14, one subject elected to stop participating after the study was already underway. One of the datasets had too much movement artifact in the genioglossus muscle recording and leak artifact in the mask pressure to use the dataset. This left 12 remaining volunteer datasets which were used for analysis of the primary endpoint.

**Expanded explanation of experimental protocol:**

The full experimental protocol included awake recordings, followed by measurements under anesthesia. In the experiment, volunteers underwent both sevoflurane and propofol anesthesia for airway recordings in a crossover-design. 18 volunteers were enrolled and 13 completed the study. A set of recordings were done with the subject awake at atmospheric pressure under 3 different PETCO2 conditions (baseline, and elevation by 4 and 8 mm Hg). Patients received either sevoflurane or propofol anesthesia, and recordings at atmospheric pressure, with CPAP, and under occlusion were measured under the 3 PETCO2 conditions. A titration algorithm for determining light and deep anesthetic levels was performed. After recordings were made under light and deep anesthesia using one anesthetic, a washout period of >40 minutes was ascertained prior to repeating the study with the second anesthetic agent. Of the 13 volunteers in which recordings were made, 12 experimental data sets were analyzed. In our measurements of reversal of anesthetic-induced airway collapsibility by elevation of end-tidal CO2, we focused our analysis on only the propofol portions of the experiment. Note that those volunteers who received propofol as the second anesthetic had > 40 minute washout time from the previous anesthetic, and an expired sevoflurane concentration of < 0.1% prior to beginning the propofol phase of the experiment.

**Analysis of occlusions:**

For every occlusion, there were a mean ± SD of 7 ± 3 breaths prior to release of the occlusion valve. To simplify Figure 2, we have only shown a few of the breaths for each condition once collapse occurred rather than all breaths. We have amended the supplemental methods to clarify that ~7 breaths were measured with each occlusion maneuver. Upon occlusion, typically after 1-2 breaths, the airway collapsed (which we identified by cessation of flow). The remaining 6 ± 3 breath attempts were with a collapsed airway. For analysis of upper airway closing pressure (primary endpoint), by definition we only used the “collapsed” breaths during occlusion. We used all breaths during the collapse period, even if the negative pulmonary pressure or genioglossus activity were increasing. Most analyses in the study were done using “collapsed” breaths. Any analyses using any other breaths were identified as such in the methods section, results section, and figure legends corresponding to those analyses.

**Selection of individualized propofol doses:**

The propofol doses in the manuscript of 3.1 ± 0.7 µg/ml and 4.3 ± 0.8 µg/ml are averages over the entire study population. However, based on our protocol, each individual volunteer had a unique pair of high and low propofol doses, with “deep” anesthesia being defined as immobility in response to a painful electrical stimulus. From a starting point of 3.7 µg/ml, propofol was carefully uptitrated and downtitrated in 50% increments to establish individualized high and low propofol doses. All volunteers were first exposed to a propofol blood target concentration of 3.7 µg/ml and a full set of data was collected. If the volunteer had no response to the electrical stimulus, this was deemed the “high” dose. The dose was then decreased by 50% to 1.9 µg/ml and the painful stimulus was applied. If motion was present, this was deemed the “low” dose. However, if the volunteer was no longer under general anesthesia, the propofol dose was uptitrated by 50% to 2.8 µg/mL. If the volunteer had a response to electrical stimulation under 3.7 µg/mL, this was deemed the “low” propofol dose, and propofol was increased by 50% to 5.6 µg/mL and loss of response to pain was attained. If this increased dose produced cessation of respiration, the dose was downtitrated to 4.6 µg/mL. Note that due to this design, all volunteers had 3.7 µg/mL as one of their propofol conditions – either high or low dose. Due to the individual nature of propofol dosing, once the doses are averaged across all volunteers, the “high” and “low” doses appear to overlap. However, as one can see from the Supplemental Table 1, in any given volunteer, the high and low doses are of course different. Most volunteer had a propofol difference of at least 0.9 µg/mL between high and low dose and had response to pain at the low dose and no response to pain with the high dose.

**Use of target-controlled infusion (TCI):**

TCI pumps use pharmacokinetic compartment models to achieve the desired plasma or effect site concentration of a drug without the anesthesia practitioner needing to administer a loading bolus or adjust subsequent infusion rates by hand. In these 3-compartment models, the central compartment is primarily plasma, while the other two compartments are a rapidly-equilibrating compartment (well-perfused tissue) and a slowly-equilibrating compartment (mainly fatty tissue). The effect site is a fourth compartment equilibrating with the central compartment. The main difference between plasmatic mode and effect-site mode of a TCI pump is the flow rate profile at the beginning of the infusion rather than the final target concentration reached. In effect site concentration mode, to rapidly attain the desired effect site concentration, an overshoot in the plasmatic concentration is permitted according to the manufacturers’ manual. The plasmatic concentration control mode does not have such an overshoot (the design is in place to protect, for example, more fragile patients). However, after an equilibration time, the plasma and effect site concentrations approach the same limit.

In our experiment, we used plasma concentration control mode, however, an equilibration time for the propofol level was ≥ 30 minutes for initiation of propofol or any subsequent anesthetic changes. Per the manufacturers’ manual, our Injectomat TIVA Agilia TCI pump uses both Marsh’s and Schnider’s pharmacokinetic models for propofol administration. For a 40 year old male who weighs 85 kg and is 178 cm tall, when a 4 µg/mL target plasma concentration is set, the Marsh model reaches equilibrium in 16 minutes and the Schnider model reaches equilibrium in 9.8 minutes [1]. Thus, our ≥30 minute equilibration time-frame will have allowed for an equilibrium between blood and effect site compartments to be reached.

**Determination of obstructive sleep apnea (OSA) and subject characteristics:**

Determination of OSA was based on the STOP Questionnaire and BMI, age, neck circumference, and gender (Bang criteria) were noted on physical exam. Specifically, patients were asked whether they snore, had witnessed apnea, or excessive daytime sleepiness in the pre-screening interview. The participants all had STOP-Bang scores corresponding to “low risk” of OSA. They were 58% male, age 24 years ± 3, height 1.7 m ± 0.1, weight 70 kg ± 13, BMI 23 kg/m2± 2. Individual patient characteristics are given in Supplemental Table 2.

As propofol anesthesia impairs the activity of the upper airway dilator muscles in a dose dependent fashion, patients without sleep apnea may develop airway obstruction under general anesthesia. See for example, the seminal paper by Isono et al [2]: the majority of normal subjects showed an airway collapse at atmospheric pressure when the upper airway muscle tone was pharmacologically mitigated or abolished.

**Results:**

**Linear mixed model parameter estimates and statistical significance**

Type III ANOVA analyses showed that for our primary endpoint (the effect of PETCO2 elevation and depth of propofol anesthesia on UA closing pressure), PETCO2 elevation, depth of propofol anesthesia, and their interaction term were significant fixed effects on closing pressure (p<0.001, p<0.001, and p=0.044, respectively). Our secondary endpoint, the effect of PETCO2 elevation and depth of propofol anesthesia on genioglossus activity) was analyzed in two ways in the manuscript. The analysis that corresponds to Figure 3 utilized all breaths, while the analysis in Supplemental Table 5 utilized only breaths during occlusions. The Type III ANOVA analyses for our secondary endpoint using all breaths showed that PETCO2 elevation, depth of propofol anesthesia, and their interaction term were significant fixed effects on closing pressure (p <0.001 for all three). Similarly, for the analysis using only occluded breaths, these same fixed effects were also significant (p<0.001, p<0.001, and p=0.001). The interaction term was included in our model in all cases. In Supplemental Table 4, we give parameter estimates for all three models used in the primary and secondary endpoints.

**SUPPLEMENTAL FIGURE 1**



**Supplemental Figure 1: Experimental setup.** Subjects wore a nasal mask connected to a high-airflow circuit with positive end-expiratory pressure (PEEP) valves on the inspiratory and expiratory limbs to deliver continuous positive airway pressure (CPAP). The airway circuit was connected to an anesthesia workstation. The inspiratory limb of the circuit included an air humidifier and an inflatable balloon occlusion valve to perform external airway occlusions by prevent inspiratory flow while allowing expiration. A 100 % CO2 tank was side-streamed to the inspiratory limb. 27-gauge stainless steel wire electrodes were inserted into the genioglossus muscle transcutaneously. A pressure catheter was inserted nasally with the tip close to the epiglottis. Capnography was performed via ETCO2 sampled from the nasal mask. A BIS monitor was placed on the forehead of the volunteers. A pneumotachograph to measure respiratory airflow was in the Y-piece of the breathing circuit. An intravenous catheter was connected to a TCI pump for delivery of propofol. A Respiratory Inductive Plethysmography device was fitted around the thorax and abdomen (not shown).

**SUPPLEMENTAL FIGURE 2**



**Supplemental Figure 3: Genioglossus** **electromyogram as a function of negative pulmonary pressure quintile.** GG-EMG and NPP measurements are pooled from 9 subjects during airway occlusion maneuvers. NPP was divided into 5 equal-sized groups with 102-103 measurements per group. NPP quintile ranges are as follows [min, max] in cm H2O: group 1 [-55.9, -31.7], group 2 [-31.6, -25.5], group 3 [-25.4, -20.2], group 4 [-20.2 -14.8], group 5 [-14.7, -4.5].Means ± SD are given. Significant difference between groups (\*; p<0.05) was determined by mixed modeling.

Abbreviations:

GG-EMG % MAX, normalized phasic genioglossus activity. NPP, negative pharyngeal pressure.

**SUPPLEMENTAL TABLE 1**

|  |  |
| --- | --- |
|  | **Propofol Concentration** |
| **Volunteer** | **Low** | **High** |
| 1 | 3.7 | 4.6 |
| 2 | 3.7 | 5.5 |
| 3 | 3.3 | 3.7 |
| 4 | 3.7 | 5.5 |
| 5 | 1.9 | 3.7 |
| 6 | 1.8 | 3.7 |
| 7 | 3.7 | 4.6 |
| 8 | 3.7 | 5.5 |
| 9 | 2.8 | 3.7 |
| 10 | 2.8 | 3.7 |
| 11 | 2.8 | 3.7 |
| 12 | 2.8 | 3.7 |
| **Mean** | 3.1 | 4.3 |
| **SD** | 0.7 | 0.8 |

**Supplemental Table 1:** The individual light and deep propofol anesthesia doses are given for all twelve volunteers.

**SUPPLEMENTAL TABLE 2**

|  |  |
| --- | --- |
|   | **PETCO2 baseline** |
| Volunteer | **light anesthesia** | **deep anesthesia** |
| 1 | 40 | 39 |
| 2 | 33 | 33 |
| 3 | 39 | 38 |
| 4 | 50 | 57 |
| 5 | 49 | 69 |
| 6 | 47 | 59 |
| 7 | 45 | 40 |
| 8 | 29 | 28 |
| 9 | 56 | 64 |
| 10 | 45 | 50 |
| 11 | 54 | 47 |
| 12 | 53 | 48 |
| **Mean** | 45.0 | 47.7 |
| **SD** | 8.4 | 12.7 |

**Supplemental Table 2:** Individual end-tidal carbon dioxide levels for each volunteer prior to supplemental carbon dioxide administration.

**SUPPLEMENTAL TABLE 3**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Subject** | **Gender** | **Age** | **Height (m)** | **Weight (kg)** | **BMI** |
| 1 | M | 20 | 1.8 | 73 | 22 |
| 2 | M | 30 | 1.7 | 64 | 22 |
| 3 | F | 24 | 1.7 | 68 | 23 |
| 4 | M | 29 | 1.8 | 74 | 24 |
| 5 | M | 22 | 1.9 | 82 | 24 |
| 6 | M | 22 | 1.8 | 86 | 26 |
| 7 | F | 28 | 1.7 | 61 | 21 |
| 8 | F | 22 | 1.7 | 54 | 19 |
| 9 | F | 24 | 1.6 | 50 | 21 |
| 10 | M | 21 | 1.6 | 70 | 27 |
| 11 | F | 23 | 1.6 | 61 | 25 |
| 12 | M | 26 | 2.0 | 95 | 25 |
| Mean ± SD | 58% male | 24 ± 3 | 1.7 ± 0.1 | 70 ± 13 | 23 ± 2 |

**Supplemental Table 3:** Individual volunteer gender, age, height, weight, and BMI

**Supplemental TABLE 4**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Parameter** | **Estimate** | **St. Error** | **Significance** | **95% CI** |
| **Pclose** | Intercept | 10.2 | 1.6 | <0.001 | 6.7 to 13.7 |
| Baseline PETCO2 | -3.1 | 0.4 | <0.001 | -3.98 to -2.2 |
| PETCO2+ 4 | -0.9 | 0.4 | .042 | -1.7 to 0.0 |
| PETCO2+ 8 | 0\* | 0 | . | . |
| light propofol | 3.2 | 0.6 | <0.001 | 2.1 to 4.3 |
| deep propofol | 0\* | 0 | . | . |
| **GG (all breaths)** | Intercept | 48 | 5.7 | <0.001 | 35 to 61 |
| Baseline PETCO2 | -19 | 1.7 | <0.001 | -23 to -16 |
| PETCO2+ 4 | -9.5 | 1.7 | <0.001 | -13 to -6.2 |
| PETCO2+ 8 | 0\* | 0 | . | . |
| light propofol | 0.0 | 1.9 | 1.0 | -3.6 to 3.6 |
| deep propofol | 0\* | 0 | . | . |
| **GG (occlusions)** | Intercept | 65 | 7.2 | <0.001 | 48 to 81 |
| Baseline PETCO2 | -28 | 2.8 | <0.001 | -34 to -23 |
| PETCO2+ 4 | -13 | 2.6 | <0.001 | -18 to -8.1 |
| PETCO2+ 8 | 0\* | 0 | . | . |
| light propofol | -0.4 | 3.2 | 0.9 | -6.6 to 5.9 |
| deep propofol | 0\* | 0 | . | . |

**Supplemental Table 4:** Parameter estimates of fixed effects are given for three linear mixed models. The dependent variable is given in the leftmost column (Pclose, genioglossus activity over all breaths, and genioglossus activity during occlusions), while the fixed effects are specified under “parameter” with their estimate, standard error, significance, and 95% confidence interval. Note that for elevation of PETCO2 by 8 mm Hg, and for deep propofol anesthesia, the parameter is set to zero (indicated by \*).

Abbreviations: PETCO2+ 4; elevation of PETCO2 by 4 mm Hg. PETCO2+ 8; elevation of PETCO2 by 8 mm Hg. GG; genioglossus. Light propofol; light propofol anesthesia. Deep propofol; deep propofol anesthesia.

**Supplemental TABLE 5**

**Table 1: Pairwise comparisons of variables for different PETCO2-level pairs while awake, under light anesthesia, and deep anesthesia**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Awake** | **Light Anesthesia** | **Deep Anesthesia** |
| **CO2 + 4 vs + 0** | **CO2 + 8 vs + 0** | **CO2 + 4 vs + 0** | **CO2 + 8 vs + 0** | **CO2 + 4 vs + 0** | **CO2 + 8 vs + 0** |
| Difference (95% CI) | P  | Difference(95% CI) | P  | Difference(95% CI) | P  | Difference(95% CI) | P  | Difference (95% CI) | P  | Difference (95% CI) | P  |
| **Ventilatory Variables** |
| **PCLOSE**  | -0.6 (-1.6 to 0.4) | 0.74 | -1.8 (-3.0 to -0.6)  | 0.01**\*** | -2.2(-3.1 to -1.3) | <0.001**\*** | -3.1 (-3.9 to -2.2) | <0.001**\*** |
| **GG-EMG**  | 11(6 to 17) | <0.001**\*** | 12(6 to 18) | <0.001**\*** | 15(9 to 20) | <0.001**\*** | 28(22 to 34) | <0.001**\*** |
| **NPP**  | -1.7(-3.0 to -0.4) | 0.03\* | -5.1(-6.6 to -3.6) | <0.001**\*** | -4.8(-6.0 to -3.7) | <0.001**\*** | -7.8(-8.9 to -6.6) | <0.001**\*** |
| **BIS**  | -0.5(-2.0 to 1.0) | 1 | 6.9(5.0 to 8.7) | <0.001**\*** | -0.8(-2.1 to 0.4) | 0.60 | -0.0 (-1.3 to 1.2) | 1 |
| **Minute Ventilation** | 2.9(1.2 to 4.5) | 0.003**\*** | 5.8(4.2 to 7.5) | <0.001**\*** | 0.7(-0.6 to 2.0) | 0.87 | 2.2(0.9 to 3.6) | 0.003**\*** | 0.5 (-0.8 to 1.8) | 1 | 1.6(0.3 to 2.9) | 0.05**\*** |
| **Duty Cycle** | 0.02(-0.20 to 0.06) | 1 | 0.02(-0.01 to 0.06) | 0.62 | -0.01(-0.04 to 0.03) | 1 | 0.01(-0.02 to 0.05) | 1 | 0.01(-0.02 to 0.05) | 1 | 0.01(-0.03 to 0.04) | 1 |
| **Peak Inspiratory Flow** | 0.11(0.00 to 0.22) | 0.17 | 0.26(0.15 to 0.37) | <0.001**\*** | 0.02(-0.07 to 0.11) | 1 | 0.09(-0.01 to 0.18) | 0.20 | 0.03(-0.06 to 0.12) | 1 | 0.10(-0.12 to 0.06) | 0.10 |

**Supplemental Table 4**: All modeled mean differences between subgroups were obtained from linear mixed modeling. Minute ventilation (L/m), duty cycle, and peak inspiratory flow (L/s) were measured during breathing prior to occlusions, while Pclose (cm H2O), GG-EMG (% MAX), and NPP (cm H2O) were measured during airway collapse. Under each anesthetic condition (awake, light propofol anesthesia, and deep propofol anesthesia), we compare elevation of PETCO2 by 4 or 8 mm Hg to baseline PETCO2, indicated as CO2 + 4 vs +0 and CO2 +8 vs +0, respectively. Values are given as mean difference (95% confidence interval). Significance is indicated by \* when p<0.05.

Abbreviations:

PCLOSE, closing pressure. GG-EMG % MAX, normalized phasic genioglossus activity. NPP, negative pharyngeal pressure. BIS, bispectral index. PETCO2, end-tidal CO2 pressure. CO2 + 0, baseline PETCO2. CO2 + 4, elevation of PETCO2 by 0, 4 and 8 mm Hg, respectively

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