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Pulmonary Gas Exchange in Response to Rebreathing-Induced Hypoxia

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Pulmonary Gas Exchange in Response to Rebreathing-Induced Hypoxia

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Abstract

Pulmonary Gas Exchange in Response to Rebreathing-Induced Hypoxia

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Rebreathing-induced hypoxia elicits changes in pulmonary gas exchange variables. **PURPOSE**: This report aims to determine the changes in pulmonary gas exchange variables in response to a rebreathing-induced hypoxia protocol in individuals who are healthy, have prediabetes, or have type 2 diabetes. **METHODS**: Fifteen participants (M: 9, age 45 ± 17 years, body mass index: 34.2 ± 8.0 kg/m^2) visited the laboratory. A 2-hour, 75 g oral glucose tolerance test was conducted during the study visit while simultaneously performing a rebreathing-induced hypoxia protocol. Venous blood samples were collected 0, 30-, 60-, 90-, and 120-min following ingestion of the glucose drink to measure plasma glucose and insulin levels. The rebreathing-induced hypoxia protocol consisted of two series of five 2-min rebreathing bouts in a low-volume, closed circuit system interspersed with two minutes of breathing room air. The first and second series of rebreathing bouts were performed within the first 30 min and 30-60 min after ingesting the glucose drink, respectively. Pulmonary gas exchange was measured using a metabolic cart. **RESULTS**: End-tidal oxygen, fraction of inspired oxygen, and respiratory rate decreased during rebreathing-induced hypoxia. Tidal volume, minute ventilation, oxygen uptake, and end-

tidal carbon dioxide increased during rebreathing-induced hypoxia. **CONCLUSION**: Rebreathing-induced hypoxia elicits changes in pulmonary gas exchange in individuals who are healthy, have prediabetes, or have type 2 diabetes.

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INTRODUCTION

Aging, increasing levels of obesity and the prevalence of a sedentary lifestyle all contribute to a rising incidence in the development of type 2 diabetes, a major modifiable risk factor involved in the development of cardiovascular diseases. Consequently, it is now predicted that one in three adults will develop diabetes by 2050, implying that a relatively large proportion of the population will become at risk for development of cardiovascular disease which is the most common cause of death in patients diagnosed with type 2 diabetes (Boyle et al., 2010). Although exercise has been shown to reduce insulin resistance and blood glucose levels, roughly 28% of the adult population diagnosed with diabetes meets the physical activity recommendations set forth by the American Diabetes Association (Resnick et al., 2006). Thus, innovative strategies such as hypoxia interventions are needed to improve the health and wellness of patients with type 2 diabetes without the assistance of exercise.

The Nobel Prize in Physiology was awarded in 2019 for work by Greg Semenza, William Kaelin, Jr., and Sir Peter Ratcliffe for their discoveries of how cells sense and adapt to oxygen availability (Lee et al., 2020; Maxwell et al., 1999; Richalet, 2021; Semenza et al., 1991). Specifically, hypoxia stimulates glucose uptake in skeletal muscle via 5' adenosine monophosphate-activated protein kinase (AMPK), the same pathway activated during exercise via muscle contraction, which acts independently of insulin (Azevedo et al., 1995; Cartee et al., 1991; Mu et al., 2001). Interestingly, patients with type 2 diabetes that were exposed to 60 minute of continuous hypoxia at ~15% fraction of inspired oxygen and resultant 92% oxygen saturation prior to a 4-hour intravenous glucose tolerance test had reduced blood glucose levels. (Mackenzie et al., 2011). With no change to insulin concentrations, this suggests an improvement in glycemic control

via the AMPK pathway in conjunction with improved insulin sensitivity. Contrary to the continuous exposure, a separate investigation exposing patients with type 2 diabetes to a single session of intermittent hypoxia, consisting a 6 minutes at a 13% fraction of inspired oxygen alternated with 6 minutes of normoxia for a total of 1 hour, resulted in improved glycemic control. Specifically, a greater decrease in glucose levels was observed immediately after this intermittent hypoxia protocol. Correspondingly, a single session of hypoxia consisting of desaturation and re-saturation to maintain an arterial saturation of 80% for approximately 70 minutes decreased blood glucose levels in overweight and obese individuals with normal baseline glucose levels (Costalat et al., 2018; Duennwald et al., 2013). Despite the success of such intermittent and continuous exposures as interventions, they remain impractical and expensive to perform regularly outside of laboratory conditions.

Rebreathing-induced hypoxia may prove to be a novel, yet simple and effective strategy to aid the reduction of postprandial glucose levels. Unfortunately, it is not an easily applicable intervention as previous research performed have manipulated the gaseous composition of air via the utilization of gas tanks, similar to hypoxia approaches. However, during short rebreathing bouts, a new approach that does not add oxygen to a closed-circuit, naturally allowing the fraction of inspired oxygen to decrease, could produce similar responses. While the benefits from breathing hypoxic air, aiding in improving blood glucose levels in patients with type 2 diabetes, are understood, it is also known that individual hypoxic ventilatory response may be vastly variable as it provides an augmentation on ventilatory activity (Pamenter & Powell, 2016). As no such method has utilized this new approach to no additional oxygen modulation, it remains to be seen what effects this may have on ventilatory activity.

Thus, this report aims to quantify variables associated with pulmonary gas exchange responses to rebreathing-induced hypoxia. We hypothesize that rebreathing-induced hypoxia will induce changes in pulmonary exchange variables in response to bouts of rebreathing-induced hypoxia.

METHODS

Participant's Information

The study included 15 participants between the ages of 25 and 65 years who were either healthy, have prediabetes, or are patients with type 2 diabetes. According to the American Diabetes Association, participants with HbA1c levels between 5.7-6.4% were considered to have prediabetes, and participants with HbA1c levels of 6.5% or greater were considered to have type 2 diabetes. Exclusion criteria included uncontrolled stage 2 hypertension (>140 mmHg systolic blood pressure or >90 mmHg diastolic blood pressure), smoking, being pregnant, having a history of cardiovascular disease or indication of cardiovascular diseases such as myocardial infarction, left ventricular hypertrophy, ischemic heart disease (or prior ischemia), stroke, and/or other vascular diseases, or having a history of diabetes or pulmonary disease. The study was approved by The Institutional Review Board at The University of Texas at Austin, 2020-05-0108, and all participants provided written consent.

Study Protocol

The laboratory visit consisted of a 2-hour, oral glucose tolerance test while simultaneously performing a rebreathing-induced hypoxia protocol. Prior to arrival, participants fasted for at least 8 hours, did not consume alcohol or caffeine for 12 hours, and abstained from intense physical activity for 24 hours before the session. Upon arrival to the laboratory, blood pressure was measured following five minutes of supine rest using an automated sphygmomanometer (Omrom Healthcare, Inc., Lake Forest, IL).

Oral Glucose Tolerance Test

The participants ingested a drink with 75g of glucose following which their blood glucose and insulin levels were measured for up to 2 hours. Venous blood samples were collected from an antecubital vein at baseline (10 min before ingestion) and 30, 60, 90, and 120 min following ingestion of a standard high glucose drink for measurements of plasma glucose and insulin concentration. For the purpose of this report, the results of the oral glucose tolerance test are not specified as the focus will be pulmonary exchange response to rebreathing-induced hypoxia in individuals who are healthy, have prediabetes, or have type 2 diabetes.

Glycated Hemoglobin (HbA1c)

HbA1c levels were determined from the blood drawn at baseline and prior to ingestion of the high glucose solution (DVC Vantage Analyzer, Siemens Healthcare GmbH, Erlangen, Germany).

Rebreathing-Induced Hypoxia Protocol

Participants remained seated after their blood pressure was recorded, throughout the duration of the rebreathing-induced hypoxia intervention and were breathing via a 4-way directional control valve (Hans Rudolph, Inc., USA) connected to a custom-made 5-liter anesthesia bag (Rusch, USA) during the first hour of the oral glucose tolerance test. First, air was added to the bag, then from minutes 5 to 25, and minutes 35-55, the rebreathing-induced hypoxia protocol consisted of two rebreathing bouts of five cycles lasting 2 minutes each interspersed with 2 minutes of breathing room air (Figure 1). In the current study, the fraction of inspired oxygen was able to be reduced by rebreathing

into a low-volume, closed-circuit system containing room air for five, 2-minute bouts of hypoxia separated by 2-minutes of breathing air in conjunction with performing a 2-hour oral glucose tolerance test for 2 series separated by 10 minutes of rest (Figure 2).

Pulmonary Gas Exchange

Breath-by-breath measures of pulmonary gas exchange such as volume of oxygen, the volume of carbon dioxide, respiratory rate, tidal volume, minute ventilation, and the fraction of inspired oxygen will be determined from a pneumotachometer (Ultima Cardio2, MGC Diagnostics, MN, USA) during the rebreathing protocol and for a duration of 5 min immediately following the ingestion of the high-glucose drink during the control visit. The pneumotachometer will be mounted between the mouthpiece and the 4-way directional control valve of the breathing circuit.

Data Analyses

For pulmonary exchange parameters, an average of the first two minutes of baseline of each phase, and the average of the final minute of each of the ten rebreathing-induced hypoxia cycles was calculated, along with the average of the final 10 seconds for fraction of inspired oxygen of each of the ten rebreathing-induced hypoxia cycles. A paired t-test was used to evaluate the effects of rebreathing-induced hypoxia vs baseline on pulmonary exchange variables. A $p<0.05$ will be considered significant. Data are presented as mean ± standard deviation (SD).

RESULTS

Participants' characteristics are presented in Table 1. Decreases in respiratory rate, end-tidal oxygen tension, and fraction of inspired oxygen occurred in response to rebreathing-induced hypoxia. Conversely, there were increases in oxygen uptake, tidal volume, minute ventilations, and end-tidal carbon dioxide during rebreathing-induced hypoxia in comparison. All pulmonary exchange variables are presented in Table 2 as mean ± standard deviation (SD).

DISCUSSION

This report aimed to quantify changes in pulmonary gas exchange variables in response to rebreathing-induced hypoxia, which may in the future serve as a more practical way to induce hypoxia and allow individuals with prediabetes and type 2 diabetes to improve blood glucose levels following a meal. We hypothesized that rebreathing-induced hypoxia would induce changes to variables associated with pulmonary gas exchange in individuals who are healthy, have prediabetes, or have type 2 diabetes. In agreement to our hypothesis, alternating bouts of breathing room air with hypoxic air resulted in increases in oxygen uptake, tidal volume, minute ventilation, and end-tidal carbon dioxide. Conversely, associated with rebreathing-induced hypoxia was a decrease in end-tidal oxygen, fraction of inspired oxygen, and respiratory rate.

Hypercapnia is a condition that results when there is an accumulation of excessive carbon dioxide in the bloodstream due to an impairment in alterations in carbon dioxide production, complications of the respiratory system, and changes in ventilatory control (Weinberger et al., 1989). Hypercapnia begins to manifest negative effects once partial pressure of carbon dioxide rises above 45 mmHg, potentially leading to an acid-base imbalance. This is important to note because the study included individuals who were either healthy, have prediabetes, or have type 2 diabetes. (Rawat et al., 2022). Our results suggest that two cycles of rebreathing-induced hypoxia limit end-tidal carbon dioxide to a rise of 42 mmHg, therefore making rebreathing-induced hypoxia a potentially safe and accessible alternative for people with prediabetes and type 2 diabetes. Should hypercapnia in the harmful range be chronic, malevolent consequences to the

cardiovascular system may develop in conjunction with epithelial dysfunction and impaired lung immunity (Csoma et al., 2022). Contrary to the present study, an increase in end-tidal carbon dioxide as a result of rebreathing-induced hypoxia was associated with increased oxygen uptake, minute ventilation, as well as increased tidal volume. Implementations of this novel technique could potentially make rebreathing-induced hypoxia a safe and accessible alternative for people with type 2 diabetes without reaching a negative partial pressure of carbon dioxide.

While indirectly related, the effects of low oxygen, hypoxia, promote similar ventilatory changes. Specifically, the effects of a single bout of interval hypoxia in patients with type 2 diabetes consisting of 6 minutes at a 13% fraction of inspired oxygen interspersed with normoxic air for a total of 1 hour resulted in small increases to pulmonary gas exchange, mediated by an increase in tidal volume. However, secondary measurements at 6 hours after the protocol ended, resulted in 19% and 17% increase to tidal volume and minute ventilation, respectively. Furthermore, no increase to respiratory rate was observed (Duennwald et al., 2013). Interestingly, end-tidal carbon dioxide exhibited a ~10% increase at 6 hours compared to baseline, and most likely was the stimulus for amplified ventilation. Yet, this increase also occurred under the placebo conditions. In the context of this study, rebreathing-induced hypoxia resulted in increased oxygen uptake (~79%), minute ventilation (118%), and tidal volume (~202%).

In agreement with our results, others report a linear increase in minute ventilation, and tidal volume, as well as a decrease in end-tidal oxygen when exposed to a

progressive increase in the partial pressure of carbon dioxide in sick patients (Read, 1967).

Contrary to the prior works, rebreathing-induced hypoxia did not result in increases in respiratory rate. Such is a surprising outcome, knowing that central chemoreceptors, including carotid bodies communicate via afferent signaling changes in pH as well as alterations in arterial carbon dioxide resulting in the stimulation of hyperventilation to aid the removal of excess carbon dioxide and therefore induce responses to both hypoxia and hypercapnia (Brinkman et al., 2022). Research investigating the effects of intermittent hypoxia as a non-pharmaceutical intervention in overweight and obese males and females found intermittent hypoxia effective in decreasing blood glucose levels while maintaining arterial saturation of 80% in conjunction with re-saturation cycles. Given that the rebreathing-induced hypoxia cycles were fixed on 2 minute intervals of hypoxic air made by decreased FiO_2 and breathing room air, the possibility has been mentioned that human hypoxic ventilatory responses could be highly variable (Costalat et al., 2018). Other research investigating the respiratory responses of diabetics to hypoxia, and hypercapnia found that 25% of their participants with type 2 diabetes displayed impaired sensitivity to hypoxia or decreased ventilatory responses to hypercapnia (Williams et al., 1984). Given that the current study had participants that were either healthy, have prediabetes, or have type 2 diabetes, it could also explain why respiratory rate did not rise as expected in response to rebreathing-induced hypoxia. Williams and colleagues also noted that a decreased ventilatory response to hypercapnia may occur as a result of impaired afferent signaling

from carotid bodies as people with type 2 diabetes may also have impaired autonomic neuropathies. Despite the study's participants having controlled type 2 diabetes, the possibility of having developed autonomic neuropathies is present, as research has shown the manifestation of such symptomology may arise before clinical presentation of type 2 diabetes (Verrotti et al., 2014). Additionally, understanding that minute ventilation remains a product of tidal volume and respiratory rate, it is possible that as minute ventilation was increased, the increase in tidal volume in response to the rebreathing-induced hypoxia intervention may have made up for the lack of increase in respiratory rate (Carpio & Mora, 2022). Despite verbal instructions and participant understanding the rebreathing-induced hypoxia protocol, few of our participants had to be reminded to breathe as they normally would. Specifically, participants explained that they felt as if they were running out of air and therefore, held their breath during the rebreathing-induced hypoxia intervention. This may help explain the lack of a total rise in respiratory rate.

CONCLUSION

The finding of this report is that rebreathing-induced hypoxia affects all pulmonary exchange variables in individuals who are healthy, have prediabetes, or have type 2 diabetes. Specifically, rebreathing-induced hypoxia resulted in decreases in end-tidal oxygen, fraction of inspired oxygen, and respiratory, rate not below levels associated with negative effects of hypercapnia. Conversely, rebreathing-induced hypoxia resulted in an increase in oxygen uptake, end-tidal carbon dioxide, tidal volume, and minute ventilation.

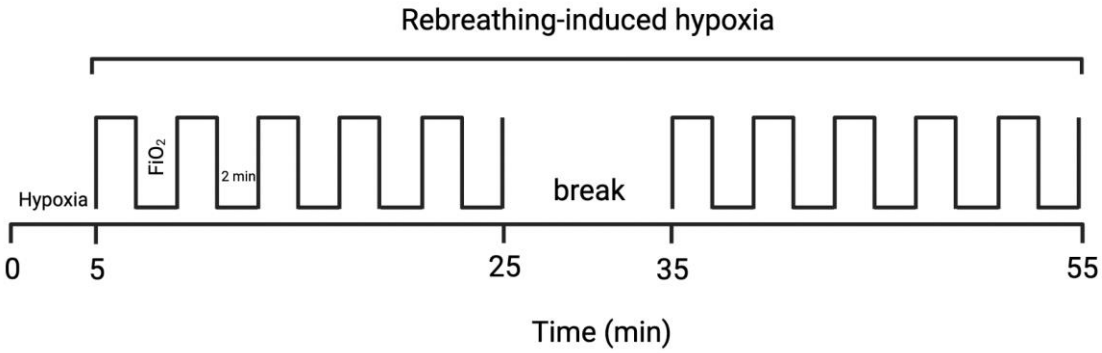

Figure 1. Rebreathing-induced hypoxia schematic

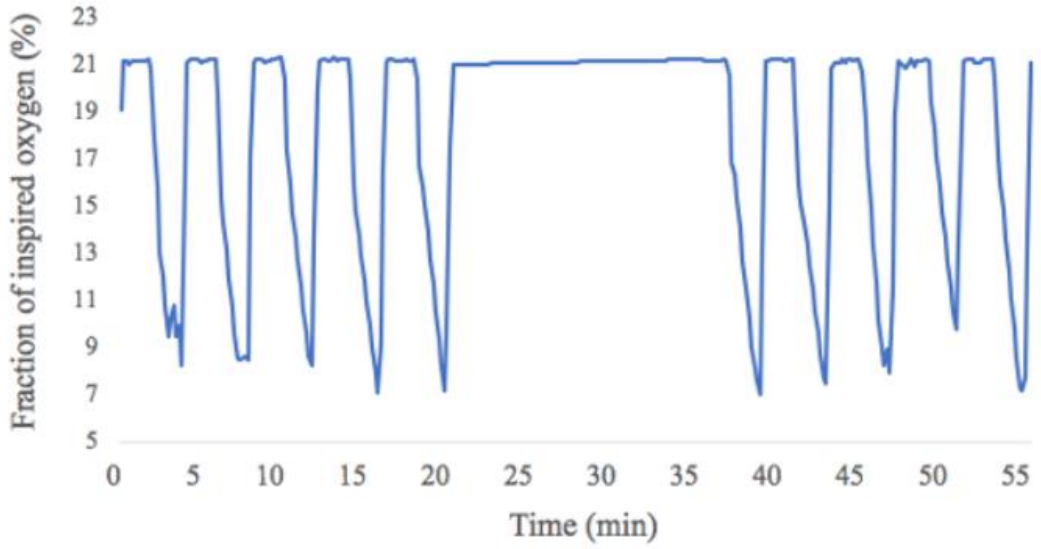

Figure 2. Ten cycles of rebreathing-induced hypoxia for fraction of inspired oxygen

Table 1. Participants' characteristics

Variables	
Sex, male/female	9/6
Age, years	45 ± 17
Body weight, kg	103 ± 23
Height, cm	174 ± 8
Body mass index, kg/m^2	34.2 ± 8.0
HbA1c, %	6.5 ± 0.8
Systolic blood pressure, mmHg	128 ± 11
Diastolic blood pressure, mmHg	80 ± 9
Heart rate, bpm	77 ± 12

Table 2. Pulmonary gas exchange variables from baseline to hypoxia

Variables	**Baseline**	**Hypoxia**
Oxygen Uptake (ml/kg/min)	3.4 ± 0.9	6.1 ± 2.7*
Respiratory Rate (breaths/min)	14 ± 5	11 ± 2*
Tidal Volume (mL)	870 ± 278	2631 ± 1027*
Minute Ventilation (L/min)	11 ± 4	24 ± 6*
End-tidal Oxygen Tension (mmHg)	105 ± 7	79 ± 10*
End-tidal Carbon Dioxide Tension (mmHg)	34 ± 6	42 ± 5*
Nadir of Fraction of Inspired Oxygen (%)	20.6 ± 1.3	12.5 ± 2.5*

*$p<0.05$: Baseline vs hypoxia

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