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Carbon dioxide rebreathing in respiratory protective devices: influence of speech and work rate in full-face masks

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Abstract

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Keywords

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Carbon dioxide rebreathing in respiratory protective devices; influence of speech and work rate in full face masks

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Carbon dioxide rebreathing in respiratory protective devices; influence of speech and work rate in full face masks

Carbon dioxide (CO_2) rebreathing has been recognised as a concern regarding respirator use and is related to symptoms of discomfort, fatigue, dizziness, headache, muscular weakness and drowsiness. Previous investigations are limited by small sample size and have not evaluated the relationship between $CO₂$ inhalation and phonic respiration (breathing during speech) in respiratory protective devices (RPDs). A total of 40 workers trained in the use of RPDs performed a graded exercise test on a cycle ergonometer that increased in workload every five minutes. During the third minute of each stage participants read aloud a prepared text. Measures of mixed expired CO_2 (PECO₂) mixed inspired CO_2 (PICO₂) and respiration were monitored. The results showed phonic respiration and low work rates contributed to significantly higher levels of CO_2 rebreathing. Aiming to reduce CO_2 exposure may result in improved wear time of RPDs.It is recommended that these findings be incorporated in technical specifications regarding human factors for RPDs.

Practitioner Summary: Carbon dioxide (CO_2) rebreathing in respiratory protective devices (RPDs) has been highlighted as key concern regarding respirator use. However the problem is relatively under researched. This paper presents novel findings on the impact of phonic respiration (breathing during speech) and $CO₂$ concentrations in RPDs.

Keywords: carbon dioxide rebreathing; phonic respiration; speech; respiratory protective devices

1. Introduction

Many researchers have been interested in determining the physiological impact of the use of respiratory protective devices (RPDs) in human wearers. In recent times carbon dioxide $(CO₂)$ rebreathing in RPDs has been highlighted as a key concern regarding respirator use. Rebreathing can occur if expired air, which is $CO₂$ rich, remains in the breathing space of the respirator after each breath. This can increase arterial $CO₂$ concentrations which can generate symptoms of discomfort, fatigue, dizziness, headache, shortness of breath, muscular weakness and drowsiness (Kloos and Lamonica 1966).

It is known that dead space (respirator volume) and hypoventilation related to breathing resistance in RPDs can contribute to $CO₂$ rebreathing. The effects of exercise, duration of respirator use, breathing technique, individual sensitivity to $CO₂$ and susceptibility to claustrophobia can also influence $CO₂$ rebreathing (ISO/TS 16976-3: 2011).

The impact of phonic respiration, or breathing during speech, on $CO₂$ levels in RPDs has not previously been evaluated. Phonic respiration occurs during exhalation and as a result decreases inhalation time (ISO/TS 16976-1: 2007). According to Boron and Boulpaep (2005) following the cessation of speech, breathing rate can increase by 25% and alveolar $CO₂$ (PACO₂) falls. Doust and Patrick (1981) proposed that hypercapnia could explain the above increase in respiration observed at the end of speech. To our knowledge no researchers have focused on the influence of speech on $CO₂$ concentrations in RPDs.

Therefore, the present study aimed to evaluate if speech and exercise workload had an impact on $CO₂$ levels in RPDs. In addition the $CO₂$ values will be compared to current respirator design standards. It was anticipated that speech, gender, body size and workload (exercise intensity) would influence $CO₂$ concentrations within RPDs. This research will lead to a better understanding of the physiological response to respirator use.

The study was conducted in two parts: a pilot study conducted at the University of Wollongong and a field study carried out at a refinery in Mount Isa, Queensland. This report outlines the results of the field study.

2. Methods

2.1. Participants

The study was approved by the Human Research Ethics Committee of the University of Wollongong/South Eastern Sydney and Illawarra Area Health Service (Reference Number: HE11/437). A total of 46 participants (one female) familiar with the use of RPDs, volunteered for the field study. Before taking part, all details of the study were explained and informed written consent was obtained from participants.

Prior to participation, participants completed a Physical Activity Readiness Questionnaire (PAR-Q) which is a self administered survey that screens individual's cardiovascular disease risk factors and symptoms (PAR-Q 2002). The State-Trait Anxiety Inventory (STAI) that assesses participants "state" and "trait" anxiety symptoms (Spielberger *et al.* 1983) was also administered. Participants were excluded if pregnant, suffering from severe illness or injury, obtained an STAI score at the ninetieth percentile or above, reported problems with claustrophobia or unable to obtain a satisfactory face fit with the RPD. In addition participants were required to be clean shaven, avoid exercise and smoking cigarettes or cigars on the day of testing.

Of this sample six participants did not meet the selection criteria for inclusion into the study, leaving a total of 40 participants (one female). The ages ranged from 19 to 58, with a mean age of 35 ($SE = \pm 1.50$). The majority of these participants were non-smokers (n=32) and 55% (n=22) reported that they were physically active or exercised on a regular basis. Information on the participants characteristics are provided in Table 1.

2.2. Equipment

A quantitative respirator fit test with a calibrated TSI Portacount Plus (TSI Incorporated; Shoreview, MN, USA) was performed to ensure that the RPD achieved an adequate face seal on the wearer. The TSI Portacount Plus uses a technique known as condensation nuclei counting (CNC) (TSI 2012). This involves the measurement of the particle concentrations outside the mask and inside the mask. The ratio of these two values is the RPD fit factor. Participants were required to obtain an overall fit factor of greater than 500 to be included in the study. This constraint was important to ensure leakage factors would not limit the results. The TSI Portacount was pre-programmed with eight sixty second exercises contained in the Occupational Safety and Health Administration (OSHA) regulations regarding quantitative fit testing protocols for RPDs (OSHA 2011). This included the following test exercises normal breathing, deep breathing, turning head side to side, up and down head movement, talking out loud, grimace (smile or frown), bending over and normal breathing.

A schematic diagram of the apparatus utilised for the exercise test is provided in Figure 1. The RPD donned was a large full face S.E.A Pty Ltd Respirator with side-mounted filter (SEA Full Face Mask-SMF-L, The S.E.A Group; Warriewood, New South Wales, Australia). The RPD was fitted with a Sundstrom SR P510-310 P3 particle filter on one side and a modular test adaptor on the other. The full face RPD was worn as shown in Figure 2.

The modular test adaptor consisted of a pressure probe which monitored inhalation and exhalation pressures. This was attached to a Validyne Pressure Transducer (Model P55D, Validyne Engineering Corporation; Northridge, California, USA). To allow for the measurement of peak inspiratory air flow (PIAF) the probe was designed to measure pressure drop in combination with the standard Sundstrom SR 510 P3 particulate filter (accuracy ±0.25% FS). The Validyne Pressure Transducer was calibrated before the assessment dates.

Two gas sampling lines were also installed in the modular test adaptor. The two gas sampling lines consisted of pneumatic valves (V1, V2). V1 and V2 (see Figure 1) were controlled to operate as one way valves and collected inspired and expired gas samples within the oronasal cup. The two sampling lines were connected externally to inhalation and exhalation accumulators. Downstream valves (V3, V4) opened one at a time, thereby isolating expired and inspired air samples during measurement. A shut off valve (V5) was used to purge the system before sampling began.

Analysis of $CO₂$ concentration in mixed expired and mixed inspired air samples was measured via an O_2 and CO_2 analyser (O_2 Cap, Oxigraph; Mountain View, USA) single channel (5-100% O_2 ; 0-10% CO_2 range). The analyser obtained air samples with a flow rate of 250 mL·min-1. The unit was calibrated at regular intervals during test procedures using certified calibration gas bottles (0% CO₂ and 5% CO₂). A Data Acquisition (DAQ) system was connected to a personal computer (PC) and collected data with 50 samples per second rate. The DAQ system performed data monitoring (such as pressure/ flow, valve control and $CO₂$ management) and data storage for further analysis.

The exercise test was performed on a stationary cycle ergonometer (Monark Bodyguard AB; Varberg, Sweden). The cycle ergonometer was calibrated before the assessment dates. Participants heart rate was measured throughout the exercise test using a Polar heart rate monitor (Polar FT1, Polar Electro; Kempele, Finland). The Modified Borg Scale (MBS) was used to measure participant's subjective level of dyspnoea (breathing discomfort). The MBS is a visual analogue scale which allows participants to rate their level of breathlessness from 0 (Nothing at all) to 10 (Maximal) accessed from the Australian Lung Foundation (2011). Scores of seven or greater (very severe) were considered termination criteria for the assessment.

The recorded outcome parameters included percentage of mixed inspired $CO₂$ (PICO₂), percentage of mixed expired $CO₂$ (PECO₂), heart rate (HR), respiratory frequency (f_R) , peak inspiratory air flow (PIAF), dyspnoea (MBS) and rate of oxygen uptake ($\dot{V}O_2$). $\dot{V}O_2$ was estimated using the leg cycling equation suggested by the American College of Sports Medicine (ACSM 2006).

$$
\dot{V}\text{O}_2 \text{ (mL·kg}^{-1} \cdot \text{min}^{-1}) = 1.8 \text{ (work rate)} / (\text{BM}) + \text{Resting } \dot{V}\text{O}_2 \text{ (3.5 mL·kg}^{-1} \cdot \text{min}^{-1}) + \text{Unloaded}
$$

cyclicing (3.5 mL·kg⁻¹·min⁻¹)

Where work rate is in kg·m·min⁻¹ and BM is body mass in kg. PIAF, f_R , PICO₂ and PECO₂ were calculated by averaging the data during 30 seconds of each measurement period (speech and no speech). The variables presented in this article were calculated across all six workloads (rest, 75 W, 100 W, 125 W, 150 W and 175 W) and the two breathing conditions (speech and no speech). Measurements of flow rates were corrected to body temperature, pressure and saturated (BTPS).

2.3. Test procedures

Exercise tests were carried out in an air-conditioned room maintained at an ambient temperature of 24° C, with an average relative humidity of 40%. Participants completed a graded exercise test on a cycle ergonometer wearing the full face S.E.A respirator. Ahead of the exercise test beginning participants sat on the cycle ergonometer for approximately five minutes while the apparatus was calibrated and resting data was collected. The test began with a two minute warm up at 50 W and a pedal rate of 60 revolutions per minute (rpm). The starting workload was 75 W or 100 W depending on the participants body size, gender or estimated fitness. The exercise protocol required a constant pedal speed of 60 rpm and increases in workload by 25 W every five minutes or after a steady state HR was reached (two heart rates within 5 beats \cdot min⁻¹). During the third minute of exercise participants read from a prepared text. Talking was discouraged during the periods before and after speech. During minute two (no speech) and minute three (speech) of each stage gas analysis and measurement of the physiological parameters (HR, MBS, PIAF) was conducted.

All participants could voluntarily halt the assessment process at any time. The test was terminated after four stages, volitional fatigue, a rating of dyspnoea of seven or greater or when the participant reached 85% of their age-predicted maximal HR (220-age). Immediately after exercise all participants were asked if they experienced any symptoms of $CO₂$ exposure, such as headache, blurred vision or dizziness. Additionally participants were allowed an active recovery period of two to five minutes (low load pedalling). The same RPD was used for each procedure. After each test the RPD was thoroughly cleaned and disinfected.

2.4. Statistical analysis

The physiological data were calculated for both breathing conditions (speech and no speech) across six workloads (Rest, 75 W, 100 W, 125 W, 150 W and 175 W). The effects of speaking and non speaking conditions on differences in $PICO₂$ and $PIAF$ at each workload were analysed using multiple paired sample *t*-tests. Linear mixed model analysis with Bonferroni test for post-hoc analysis was conducted to determine the significance of the effects of $\dot{V}O_2$ and the experimental conditions, speech and no speech, on PICO₂. A significance level of $p < 0.05$ (two tailed) was used for all statistical analysis. All analyses were completed using Statistical Package for the Social Sciences (SPSS) version 19.

3. Results

Of the 46 volunteers, 13% (n=6) did not meet the selection criteria for inclusion into the study. Five participants were excluded from participation at the level of the PAR-Q form and one due to equipment failure. All participants passed a quantitative respirator fit test (>500 protection factor) with a Portacount. Data for the remaining 40 participants who completed all phases of the test are presented in Table 2.

The duration of the exercise test, including warm up varied from 8-22 minutes. Within this, 12 participants (30%) did not reach 85% of their age-predicted maximal HR. Reasons to stop the exercise test before target HR was reached included lower limb fatigue (n=6), end of exercise protocol (n=3), severe breathing discomfort or dyspnoea (n=2) and general fatigue $(n=1)$.

3.1. Speech (phonic respiration)

The mean $PICO₂$ values that occurred during periods of speech and no speech for both rest and exercise are shown in Table 3. Paired *t* tests were carried out to compare differences in $PICO₂$ between the two conditions. A significant difference in $PICO₂$ between periods of speech and no speech occurred at rest, 75 W, 100 W, 125 W and 150 W. Although there was a relationship at 175 W, it did not achieve significance.

In general PICO₂ levels were below 2% in periods without speech. However during speech, $PICO₂$ was observed to often exceed this, above all at rest. An elevation of $PICO₂$ above 3% (100 times atmospheric concentrations) was experienced by three participants at rest (speech). One in three participants $(n=11)$ were exposed to PICO₂ greater than 2% during periods of work and speech (75 W, 100 W and 125 W). In the absence of speech PICO₂ concentrations were observed to decrease, especially with each increase in workload. The lowest average PICO₂ (0.97%) occurred at a mean $\dot{V}O_2$ of 28.7 mL·kg·min⁻¹, no speech. No participants reported symptoms of headache, blurred vision or dizziness.

3.2. Peak inspiratory air flow

The mean PIAF for all workloads during periods of speech and no speech is displayed in Table 4. A paired samples *t* test was conducted to compare PIAF and the two breathing conditions (no speech and speech) across the six exercise workloads (rest, 75 W, 100 W, 125 W, 150 W and 175 W) (alpha was set at 0.05). There was a significant difference in PIAF between periods of speech and no speech at rest, 75 W, 100 W, 125 W and 175 W. Although there was a relationship at 150 W, it did not achieve significance.

The highest PIAF scores were seen during speech. The maximum mean PIAF was 323.50 L·min-1 and occurred at 175 W during speech, whereas the lowest mean PIAF was 80.50 L \cdot min⁻¹ occurred at rest and during no speech.

3.3.Oxygen uptake

The mean PICO₂ levels as a function of mean $\dot{V}O_2$ during speaking and non speaking periods is displayed in Figure 3. The highest mean ergonometer power setting equivalent to $\dot{V}O_2$ 33.2 mL·kg⁻¹·min⁻¹ gave rise to PICO₂ of 1.0% (no speech) and 1.4% (speech). It appears with increased $\dot{V}O_2$, the RPD is more efficient in the removal of dead space CO_2 .

Linear mixed model analysis was conducted to assess the effects of $\dot{V}O_2$ and the experimental conditions, speech and no speech on PICO₂. There were five levels of $\dot{V}O_2$ corresponding to the following groups: rest (n=40), 75 W (n=19), 100 W (n=40), 125 W (n=36) and 150 W (n=22). Note that 175 W was not tested due to unsatisfactory sample size. Statistical significance was set at an alpha level of 0.05.

Without speech, the effect of $\dot{V}O_2$ on PICO₂ was significant, F (1, 4) = 19.8, p=0.00. Similarly, interactions between speech and $\dot{V}O_2$ had significant effects on PICO₂, F (1, 4) = 25.7, $p = 0.00$. Post-hoc tests were conducted to examine all pairwise contrasts using the Bonferroni adjustment. Since this involved five pairwise contrasts for each workload (excluding 175 W due to small sample size) the critical alpha level to be used for these contrasts was 1/5 times 0.05, that is, a critical α of 0.2. Of the five contrasts without speech, level one (rest) differed significantly from all others and level 5 (150 W) differed significantly from level 1 (rest) and level 3 (100 W). However, level 2 (75 W) did not differ significantly from 3 (100 W) or 4 (125 W) ($p<0.05$). Similarly during speech, level one (rest) differed significantly from all others. Level 3 (100 W) did not differ from level 2 (75 W), level 4 (125 W) and level 5 (150 W). This reflects that CO2 rebreathing is reduced once a higher $\dot{V}O_2$ is obtained with exercise. However the difference between PICO₂ vs. small increments in $\dot{V}O_2$ with exercise is less significant.

3.4. Effects of mixed expired carbon dioxide

On average PECO_2 appeared to be higher during periods without speech. The highest mean PECO_2 was 5.8% and occurred at 75 W (speech) and 100 W (no speech). However the lowest mean PECO_2 was 2.9% and occurred at rest (speech).

3.5. Dyspnoea

Dyspnoea (MBS) scores during speech and no speech periods are shown in Table 2. No dyspnoea was reported during resting conditions. Ratings of dyspnoea was reported to be somewhat severe (4) or higher by 92% (n=12) of participants at 150 W (speech) and 100% (n=3) at 175 W (speech). Breathing discomfort was reported to rise during both increases in exercise effort and periods of speech. Due to the opposing effects of these variables on $PICO₂$ the relationship between $PICO₂$ and dyspnoea did not produce a significant result in this study.

3.6. Heart rate

HR was on average 2.9% higher during speech conditions than no speech at the same

workload. Therefore speech appears to increase the work of breathing (energy expenditure). This effect appeared to decrease as workload increased (175 W excluded).

3.7. Breathing frequency

Overall, there was a reduction in mean f_R during speech. During speech f_R decreased by 23.5% at rest. However progressive increases in exercise workloads caused participants to speak fewer words from the text and f_R gradually increased. Consequently f_R during speech and non speech periods at 175 W (peak exercise) was comparable.

4. Discussion

Increased levels of $CO₂$ rebreathing in RPDs can have a profound effect on the respiratory system and is a concern regarding respirator use (ISO/TS 16976-3: 2011). A literature review by NIOSH (1976) indicates 1% inspired $CO₂$ is associated with respiratory stimulation such as increased f_R , alveolar CO₂ and $\dot{V}O_2$. This current study demonstrates that periods of speech in RPDs cause an increase in inspired $CO₂$ well above the normal concentration found in atmospheric air (0.03%) (Williams 2010). Almost one in three participants inspired $CO₂$ concentrations 2% or higher during periods of speech at sedentary to low work rates. These findings suggest prolonged speech can contribute to $CO₂$ surpassing current respirator design standards that specify inspired $CO₂$ should not exceed 1% for more than one consecutive minute when testing RPDs (AS/NZS 1716: 2003). This specification is also applied in the Occupational Safety and Health Standards of OSHA: 1910.134 "Respiratory Protection" and European Standards: EN 13274-6: 2002 for respirator classification.

Similarly, Roberge *et al.* (2010) examined the physiological impact of N95 filtering face piece respirators. Ten adults (seven women) conducted two 60 minute treadmill assessments at very low workloads walking at 2.74 km·hr⁻¹ (1.7 miles·hr⁻¹) and 4.02 km·hr⁻¹ (2.5 miles \cdot hr⁻¹) while wearing the RPD. Data collected showed that dead-space CO_2 ranged from 2.5-3.5% $CO₂$ which is significantly above OSHA's ambient workplace standards. Roberge *et al.* (2010) concluded that even though the RPD did not impose any significant physiological burden on participants, $CO₂$ retention was a possibility due to elevated transcutaneous CO_2 (equivalent to arterial CO_2) levels. On a similar note, although no symptoms of CO_2 retention were recorded in this study, the increases in CO_2 during speech were sufficient enough to impact the participant.

There has also been concern that exercise compounds $CO₂$ rebreathing in RPDs due to increased metabolic CO_2 production (Williams 2010). In the current study PICO₂ was shown to be inversely related to exercise. This demonstrated that the large full face S.E.A Pty Ltd Respirator was efficient in the removal of dead space $CO₂$ at higher work rates. These findings support research conducted by Kloos and Lamonica (1966) and Luria *et al.* (2004) who found low work rates during RPD use was associated with $CO₂$ accumulation. A previous study by Luria *et al.* (2004) attributed this to a rise in ventilation and lower dead space during higher exercise efforts.

We also noted that breathing frequency and positive pressure in the mask decreased each time speech was added. This also suggests speech produces a reduction alveolar respiration without a change in metabolic rate which tends to increase $CO₂$ concentrations in RPDs (ISO/TS 16976-3:2011).

The International Organization for Standardization (ISO) prepared a technical report on the effects of hypercapnia and the impact of $CO₂$ concentrations on respirator use (ISO/TC 16976-3.2: 2010). ISO (2010) specified that increased concentrations of $CO₂$ in the breathing space of a RPD may generate dyspnoea which causes the user to remove the device. ISO (2010) concluded that aiming to reduce $CO₂$ exposure in the breathing space of a RPD is important to improve the wear time of RPDs in the workplace. The results of the present study indicate the impact of speech, low work rates and respirator use needs to be evaluated. We suggest that the findings in this study be incorporated in technical specifications regarding human factors for RPDs.

Sensitivity to $CO₂$ is a variable that may have confounded the effects of $CO₂$ rebreathing in the present study. It should be acknowledged that the level of response to $CO₂$ rebreathing varies considerably from person to person. For instance research by Love *et al.* (1979) and Takahashi *et al.* (2000) found when $CO₂$ was added to inspired air all participants increased their respiration but the degree of this response varied considerably.

There was a small increase in HR noted during speech. These effects demonstrate that there is an increase in physical exertion during speech and RPD use. Therefore speech may limit physical performance while wearing RPDs.

In this study, the process of speaking and exercise was enough to cause symptoms of breathing discomfort. Therefore the present study could not attribute any physiological symptoms of dyspnoea to $CO₂$ rebreathing.

Also, participants were more likely to pause from speaking at higher workloads due to the breathing requirements required for exercise. This would improve oxygen delivery to the participant wearing the RPD and decrease the level of $CO₂$ rebreathing. This can potential confound the results.

It is also important to note exercise intensities in this study were only set at low to moderate workloads. Therefore differences in P_{ICO_2} during maximal exercise, where $CO₂$ production can exceed 4 L \cdot min⁻¹ (ISO 16976-3: 2010) cannot be compared and limits the interpretation of these results.

A further limitation of the study was the underrepresentation of women and smallmedium body surface area (BSA) groups. Differences in gender and BSA might influence $CO₂$ production and sensitivity to $CO₂$ exposure. Future research could overcome this by analysing women and different BSA groups separately.

5. Conclusion

Overall, the results of the study indicate speech and low work rates significantly increase $CO₂$ rebreathing in RPDs. Based on Australian respirator design standards it is evident speech could contribute to inspired CO₂ exceeding the maximal allowable concentrations in inspired air. However, the impact of gender and body size on $CO₂$ levels could not be ascertained. The implication of these findings is that high $CO₂$ concentrations in full face RPDs may be linked to wearer discomfort and contribute to reduced tolerability and wear time of the device. Since many occupations require workers to communicate while wearing RPDs these findings must be taken into consideration. It is recommended that the findings in this study be considered in the design and use of RPDs.

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Table 1. Characteristics of the field study participants

	Median Min		Max
Age (years)	34	19	58
Weight (kg)	89.5	58.0	128.0
Height (m)	1.77	1.67	1.92
BSA (m^2)	2.09	1.67	2.41
State Anxiety Score (%)	27	20	50
Trait Anxiety Score (%)	30.5	20	47

M, Mean, SE, Standard Error of the Mean, PICO₂, Percentage of Inspired Carbon Dioxide, PECO₂, Percentage of Expired Carbon Dioxide, HR, Heart Rate, f_R , Breathing Frequency, PIAF, Peak Inspiratory Air Flow, MBS, Modified Borg Dyspnoea Scale, BTPS, Body Temperature & Pressure Saturated. Note. *=Statistical significance (p≤0.05) from paired samples *t*-test **PIAF is given in BTPS and rounded to the nearest 0.25.

	No Speech		Speech			
	ΙVΙ	SE	IV.	SE		df
Rest		0.06	$2.1*$	0.08	7.75	38
75 W		0.03	-5*	0.06	6.07	. O
100 W		0.04	$.6*$	0.06	6.07	
125 W		0.03	$5*$	0.06	6.57	33
150 W	0.1	0.03	$.4*$	0.05	4.90	
175 W		0.04		0.03	293	

Table 3. Mean carbon dioxide inspired at rest and exercise for conditions of no speech and speech

M, Mean, SE, Standard Error of the Mean. Note. *=Statistical significance (p≤0.05) from paired samples *t*test.

		No Speech		Speech		
	М	SE	M	SE		df
Rest	80.50	2.51	$125.75*$	4.87	2.85	39
75 W	150.00	2.37	225.00*	4.56	5.27	34
100W	172.75	3.24	247.25*	4.30	4.14	39
125 W	201.50	3.16	268.75*	4.07	4.47	39
150 W	232.25	4.88	305.25	5.62	2.05	39
175 W	227.75	5.03	323.50*	7.34	.78	39

Table 4. Mean peak inspiratory air flow at rest and exercise for conditions of no speech and speech

M, Mean, SE, Standard Error of the Mean. Note. *=Statistical significance (p≤0.05) from paired samples *^t*-test .