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ABSTRACT (230/250 words)

Maximal static dry, i.e., on land, apneas (breath-holds) result in severe hypoxemia and hypercapnia and have easy-going and struggle phases. During the struggle phase, the respiratory muscles involuntarily contract against the closed glottis in increasing frequency and magnitude, i.e., involuntary breathing movements (IBMs). IBMs during a maximal static apnea have been suggested to fatigue respiratory muscles, but this has yet to be measured. Thus, the purpose of this study was to quantify respiratory muscle strength pre- and post-apneas in an elite, world champion, world record holding apneist. To do so, maximal inspiratory and expiratory pressure maneuvers (MIP and MEP, respectively) were performed pre- and post-apnea protocol which included 3 preparatory apneas with 2.5 min rest. All preparatory apneas were ended after the participant reported 7-10 IBMs. Next, he performed 3 maximal static dry apneas with 5 min rest in between. The participant had maximal apneas lasting 363, 408, and 460 seconds. Including preparatory apneas, the participant's total apnea duration was 33.4 min in 57.0 min. Following the apnea protocol, i.e., pre vs. post, there was no change in MIP (-124.2 vs. -123.6 cmH_2O) or MEP (259.4 vs. 262.5 cmH_2O). These data, albeit in a single individual, suggest that respiratory muscle strength is not impacted by maximal static breath-holds. This could be the result of training and/or be a feature of this individual that allows him to excel in this sport.

NEW AND NOTEWORTHY (73/75 words)

Previous work has suggested that respiratory muscle fatigue may result from maximal breath-holds, but this has not been measured. We measured respiratory muscle strength pre- and post-maximal apneas in a world champion breath-hold diver. We found no change in respiratory muscle strength following a series of apneas. This may be an adaptation of the diver's training or a feature of their physiology that allows them to be successful in this physiologically challenging sport.

INTRODUCTION

The physiological response to a maximal breath-hold is characterized by two distinct phases (1). The first phase is the “easy-going phase” and is a period of physiological quiet as O_2 is plentiful, CO_2 remains low, the glottis is closed, and the respiratory neural drive to breath is voluntarily suppressed. However, throughout this phase the urge to breathe gradually increases as a result of increasing CO_2 from metabolism and decreasing O_2 from use and lack of breathing until a physiological breakpoint is reached. Once this point is reached the individual enters the “struggle phase.” During this phase the respiratory control center overriding the individual’s voluntary suppression of respiratory muscle contractions and neural output to the respiratory muscles increases and the muscles of breathing, both inspiratory and expiratory, begin to contract, i.e., involuntary breathing movements (IBMs), against the closed glottis. These contracts occur in a crescendo pattern, i.e., increasing progressively for the duration of the struggle phase until breathing resumes (2).

Only one previous study has quantified and characterized respiratory muscle pressure development during a maximal static breath-hold in elite apneists (2). Cross and colleagues reported that changes in transdiaphragmatic pressure (P_{DI}) were four-fold greater at end-apnea compared to during supine rest (2). Additionally, the pressure-time indexes for the diaphragm and expiratory rib cage muscles (approximation of the muscles' energetic demand) during the struggle phase increased by 5- and 15-fold, respectively (2), in excess of their respective "critical" threshold values (3, 4). The critical threshold refers to a specific pressure-time index at which task failure, i.e., fatigue, occurs (3, 4). Accordingly, the data from Cross et al., (2) suggests that respiratory muscle fatigue occurred as a consequence of a maximal apnea, but these measures have yet to be made in apnea divers.

We recently had the unique opportunity to make measures in an elite, world champion, and world record-holding apneist. In this individual, we sought to determine whether or not the respiratory muscles were fatigued, i.e., strength was reduced, as a result of a series of

preparatory and maximal apneas. To do so, we quantified respiratory muscle strength pre- and post-apnea protocol. We hypothesized that respiratory muscle strength would be reduced following maximal breath-holds.

METHODS

Ethical Approval and Screening

The participant was male and 28 years old at the time of participation. He provided written and oral informed consent prior to participating in the experiments. The study was approved by the Human Research Protection Program at Northern Arizona University and the Ethical Committee of the Faculty of Kinesiology, University of Split in Split, Croatia. The study was conducted in Split, Croatia.

Pulmonary Function

Spirometry, including slow and forced vital capacity maneuvers, was assessed using a computerized spirometer (CPFS/D Spirometer; MedGraphics; St. Paul, MN, USA) and conducted and reported per societal standards (5) and using appropriate predictive equations (6).

Experimental Design

The participant visited the laboratory on a single occasion and followed the timeline reported in **Figure 1**. Upon arrival, the participant's anthropometrics, including height, mass, and body composition was assessed via a bioimpedance scale (Tanita BC 418; Tokyo, Japan). Spirometry was then performed. Next, the participant had electrodes placed on the 7-8th intercostal space, bilaterally, to assess the electrical activity via electromyography (EMG) of the right and left hemispheres of the diaphragm, as before (7–9). A forehead sensor was placed over the left eye to provide a peripheral estimate of arterial oxygen saturation (SpO_2). Then, respiratory muscle strength was assessed by measuring maximal inspiratory and expiratory muscle pressure generation (MIP and MEP, respectively). The participant then performed three preparatory apneas and 3 maximal apneas. Finally, MIP and MEP were assessed 10 min following the conclusion of the final apnea.

Apnea Protocol

The apnea protocol was developed to increase the duration of the maximal apneas and more closely mimic what the individual may do in preparation for a competition. The diver began the protocol by resting quietly in the supine position in a dimly lit, climate-controlled laboratory. The protocol included 3 preparatory apneas; there was a 2 min 32 second recovery period between apneas 1 and 2 and 3 min and 9 seconds between apneas 2 and 3. The first apnea was performed at the end of a normal exhalation, i.e., at functional residual capacity, and concluded after the individual experienced 9 involuntary breathing movements. IBMs during the preparatory apnea were monitored and reported by the participant. The remaining two preparatory apneas were performed after a complete inhalation to total lung capacity and, again, concluded after 10 IBMs were reported. After 5 min and 43 seconds of rest the individual performed the first of three maximal apneas. The participant was instructed to perform the maximal apneas in the manner that they would do so during a competition and were allowed to perform glossopharyngeal insufflation ("lung packing") ad libitum. The recovery periods between the maximal apneas were 5 min 56 seconds and 6 min and 9 seconds, respectively.

Peripheral Estimate of Arterial Oxygen Saturation and Heart Rate

SpO_2 and heart rate were quantified using a pulse oximeter (Nellcor Oximax N-600, Tyco, Mansfield, MA, USA) and forehead sensor (Oximax Max-Fast). Data were measured continuously for the duration of the apnea protocol. Although we did not make direct measures of arterial oxygen saturation, previous work has demonstrated very strong agreement (R^2 = 0.978) between direct measures and estimates using the make and model of this device used in this study between a wide range of arterial oxygen saturations (99.5 to 63.5%) (10).

Respiratory Muscle Pressure

To assess maximal respiratory muscle strength the participant performed MIP and MEP maneuvers according to societal standards (11). Briefly, a rubber mouthpiece (Hans Rudolph Inc., Shawnee, KS, USA) was placed over the opening of a 3-D printed custom device. This device was a capped cylinder that was 7.5 cm long with a 3 cm internal diameter. It had a port (opening) on the side where air could flow into and out of the cylinder distal to the participant's mouth and two ports with luer locks connected to them. One luer lock was connected to connected to a differential pressure transducer via tygon tubing and the other luer lock was either capped (during MEP) or had a small piece of tubing connected to it to provide a slow leak (during MIP). Voluntary pressure generated inside of the cylinder was taken to reflect maximal respiratory pressure generating ability. Pressure was measured with a differential pressure transducer with a range of ±352 cmH_2O (TSCDRRN005PDUCV, Honeywell International Inc., NJ, USA) that was calibrated using a digital manometer before each visit.

The MIP maneuver was done by having the participant exhale completely to residual volume. Upon arrival at residual volume, the participant was instructed to cover the lateral distal port on the cylinder with their finger, and then inhale maximally for 3-5 seconds. The MEP maneuver was done by having the participant inhale completely to total lung capacity. Once the lungs were completely full, the participant was instructed to cover the lateral distal port on the cylinder, and exhale maximally for 3-5 seconds. During the MEP maneuver, an investigator applied pressure to the participant's cheeks to prevent engagement of buccal muscles during the maneuver. Each measurement was performed a total of 5 times and the participant was strongly encouraged to give their best effort and were provided visual numerical feedback on the pressure generated for each effort. The three closest maneuvers (coefficient of variations were between 0.6 and 2.6%) were taken as the MIP and MEP.

Data Acquisition and Analysis

Data were acquired at 1000 Hz using PowerLab (PowerLab 16/35; ADInstruments; Colorado Springs, CO, USA). Diaphragm electromyographical data were collected using an Octal Bioamp (FE238; ADInstruments; Colorado Springs, CO, USA). The raw EMG data were imported into MATLAB (v2024a, The MathWorks Inc., Natick, MA, USA) and then processed using a Fast Fourier Transformation and a second-order Butterworth bandpass filter between 20 and 450 Hz to increase the signal-to-noise ratio. In addition, filtering was performed on the raw data with a root mean square (RMS) filter. The RMS envelope calculation was performed with fully overlapped windows so that the sampling rate of the RMS was identical to the sampling rate of the acquired data. Using these filtered and smoothed data we were able to observe and count the number of IBMs that occurred during the struggle phase. An IBM was considered present when the signal increased above the baseline established during the easy-going phase. All data were visualized on LabChart Pro (v8.1.16; ADInstruments; Colorado Springs, CO, USA). MIP and MEP were taken as the 1-second average pressure that surrounded the peak pressure achieved.

RESULTS

Apnea Personal Records, Anthropometrics, and Spirometry

This individual has personal records of an 8 min and 25 second static wet apnea, a maximal depth of 84 m in the constant weight discipline, a maximal dynamic apnea with fins of 234 m, and 112 m walked underwater on a single breath (world record). The participant is 175 cm tall with a mass of 64.1 kg and a body mass index of 21.2 kg/m^2. Their body fat percentage was 11.6%. The participant had normal spirometry with a forced vital capacity of 5.95 L (108% predicted), forced expired volume in 1-second of 4.45 L (98% predicted), and a forced expired volume in 1-second to forced vital capacity ratio of 75% (90% of predicted).

Apnea Durations, Heart Rate and SpO_2, and Diaphragm EMG

The durations of the apneas are displayed in **Figure 2**. The maximal apneas lasted 363, 408, and 460 seconds. At the end of the each of the preparatory apneas, heart rate was 52, 62, and 65 bpm and SpO_2 was 59.4%, 80.5%, and 83.7%. At the end of the each of the maximal apneas (last ~10 seconds), heart rate was 68, 69, and 88 bpm and SpO_2 was 65.4%, 47.9%, and 30.0%. Interestingly, there is a steep decline in SpO_2 at the onset of the struggle phase (**Figure 3**). Based upon the diaphragm EMG data, the participant had 13-29 IBMs, as observed via diaphragm EMG, in each of the maximal apneas. **Figure 3** displays a tracing of diaphragm EMG, SpO_2, and heart rate during the participant's final, maximal apnea. The spikes in EMG reflect an IBM.

MIP and MEP

MIP and MEP data are displayed in **Figure 4**. MIP was -124.2 and -123.6 cmH_2O pre- and post-apnea protocol. MEP was 259.4 and 262.5 cmH_2O pre- and post-apnea protocol. As illustrated in the figure, there was no change in MIP or MEP following the apnea protocol.

DISCUSSION

We aimed to determine whether or not respiratory muscle strength was negatively impacted by maximum breath-holds in an elite apnea diver. We hypothesized that MIP and MEP would decrease following our designed apnea protocol. However, we found that MIP and MEP were unchanged despite many IBMs in the presence of severe arterial hypoxemia and, presumably, hypercapnia.

Respiratory muscle contractions (IBMs) begin at the onset of the struggle phase and the frequency and magnitude of these contractions increase for the duration of the maximal apnea (1, 2, 12). Previous work has demonstrated that both inspiratory (diaphragm and external intercostals) and expiratory (abdominals and internal intercostals) muscles are involved in these contractions (2, 12–16). In the present study we were unable to identify expiratory muscle participation during IBMs, but surface EMG of the diaphragm confirms the diaphragm was active during IBMs. We did not observe a decrease in MIP and MEP pre- and post-apnea protocol. Importantly, our measures of respiratory muscle strength provides us with information about *global* inspiratory and expiratory muscle strength vs. specific, individual respiratory muscles. Previous work has suggested that accessory muscles of respiratory become progressively more involved in contractions during the struggle phase of a maximal apnea to, presumably, delay or prevent fatigue of the primary muscles of respiration, i.e., the diaphragm and intercostals (17–19). Unfortunately, our employed methodology does not allow us to determine whether or not this occurred in our participant, but warrants future study. It is possible, that the absence of fatigue was the result of this adaptive breathing strategy whereby the fatigue was “distributed” across multiple respiratory muscles, but was insufficient to elicit a measurable decrease in MIP and/or MEP. In other words, perhaps the diaphragm was fatigued, but the inspiratory accessory muscles were not and so global inspiratory muscle strength was unaffected by our apnea protocol.

Nevertheless, it is somewhat surprising that we saw no change in MIP and MEP as a result of our apnea protocol based on prior work (2). Cross and colleagues quantified the pressure-time index of the diaphragm and the rib cage muscles during IBMs during the struggle phase of a maximal apnea (2). This work (2) reported that the diaphragm had an increase in its pressure-time index of 5-fold and exceeded the previously established threshold for diaphragm task failure, which would occur as a consequence of fatigue (3), for the final 40% of the struggle phase. Similarly, the magnitude of change in the pressure-time index of the rib cage muscles increased by 15-fold and exceeded the previously established threshold for task failure (4), for the final 20-30% of the struggle phase. Importantly, our methodology did not allow us to calculate the pressure-time index of these respiratory muscles during the struggle phase, but our diaphragm EMG data clearly demonstrate the presence of increasing magnitude of IBMs. The work that established these critical thresholds for task failure of the diaphragm (3) and rib cage muscles (4) did so in long duration (~45 min) of resistive breathing. This is substantially longer than a maximal apnea and, certainly, contained more respiratory muscle contractions than a maximal apnea. If we assume that our participant followed a similar pattern as those of Cross et al., (2) then we believe we could have seen respiratory muscle fatigue giving the difference in the arterial blood gas environment experienced by our participant and those of previous work (3, 4).

Our participant experienced severe arterial hypoxemia (SpO_2 of 65 to 30%) by the end of the apneas. Similarly, our participant likely experienced hypercapnia during their maximal breath-holds. Unfortunately, we did not have measures of arterial PCO_2 in the present study, but previous work has shown arterial PCO_2 to increase 10-15 Torr by the end of the breath-hold of shorter duration (mean of 316 seconds vs. 1,229 seconds combined apnea in the present study) (21). Both hypoxemia and hypercapnia can contribute to respiratory and skeletal muscle fatigue. Specifically, arterial hypoxemia has been shown to expedite and exacerbate respiratory muscle fatigue during spontaneous breathing, i.e., exercise (22–25). Likewise, hypercapnia has been

shown to expedite and/or exacerbate respiratory muscle fatigue during exercise (26) and during various breathing tasks, i.e., pressure-threshold loading or resistive breathing (17).

Given the above results from spontaneously breathing humans, the physiological milieu during a maximal breath-hold could have contributed to respiratory muscle fatigue. There are some possible reasons for the lack of fatigue in this individual. First, it is possible that there is a training effect, both in-season and over many years, that has made the participant's respiratory muscles severely fatigue-resistant. Parallel to this, fatigue-resistant respiratory muscles could be a feature of the participant's status as an elite apnea diver. In other words, it is possible that the diver's apnea duration is dependent upon their respiratory muscles being resistant to fatigue. Future work should examine freedivers with varying maximal breath-hold durations to test this hypothesis. Second, it is possible that our participant did not have severe enough IBMs to cause respiratory muscle fatigue. Anecdotal reports suggest that some elite apneist experience few or no IBMs or that their IBMs are visually undetectable. Without a measure of rib cage movement or respiratory pressures we cannot comment on the magnitude, i.e., the pressure swings generated by the respiratory muscles, of our participant's IBMs. Third, it is possible that our participant was afforded too much rest time between maximal apneas for fatigue (central or peripheral) to arise, i.e., there was sufficient time for complete recovery. However, pervious work has demonstrated that respiratory muscle fatigue (peripheral) persists for at least 30 minutes following dynamic exercise (9). It is possible that the participant experienced central fatigue that was resolved by the time they performed MIP and MEP maneuvers following the apnea protocol (~10 min). It has been suggested that recovery from central fatigue can reach ~80% recovered after only 4-5 minutes (27). Importantly, our breath-hold protocol was designed to result in the longest apnea durations possible so as to incur as many IBMs as possible. Fourth, it is possible that our methodology did not allow us to observe respiratory muscle fatigue. We discuss these methodological limitations below.

Limitations

There are several methodological limitations that are worth discussing. First, we employed measures of respiratory muscle strength that required the participant to voluntarily contract the muscles. Reliance on voluntary maneuvers can also lead to participant fatigue where the individual is simply "tired" of doing the maneuvers and does not give maximal effort. This would typically manifest as observing fatigue when it was not present so is not relevant in our experiment. There can also be a learning effect for these voluntary maneuvers. We did not provide the participant with a dedicated familiarization period and, thus, a lack of fatigue could have actually been a learning effect. However, in our experiment, we had the participant perform 5 MIP and 5 MEP maneuvers and selected the 3 closest maneuvers. We observed a very narrow range of values on these 3 maneuvers as illustrated in (**Figure 4**). The spread of values across MIP and MEP at both time points was 0.6 to 2.6% so we do not believe there was a learning effect in our study. Future work should employ methods to remove the voluntary component of respiratory muscle pressure generation, i.e., phrenic nerve stimulation and/or thoracic root nerve stimulation (7, 9). Finally, had we inserted balloon-tipped catheters into the esophagus and stomach we could have quantified pressure generated by the diaphragm (gastric pressure – esophageal pressure) and abdominal muscles (gastric pressure) during voluntary MIPs and MEPs. This would have allowed us to parcel out primary muscles of respiration vs. total respiratory muscles and observed how this changed pre- and post-apneas. For example, it is possible that the diaphragm was fatigued, but the rib cage muscles were able to compensate for this and total pressure generated was unchanged.

Conclusions

We found that respiratory muscle function was unchanged by apneas despite significant apnea duration (total = 33.4 minutes) and severe arterial hypoxemia (SpO_2 as low as 30%) in an elite, world champion, and world record holding breath-hold diver pre- and post-apneas. These

findings could be the result of training and/or a feature of what makes this individual so successful in this sport.

REFERENCES

1. **Lin YC**, **Lally DA**, **Moore TO**, **Hong SK**. Physiological and conventional breath-hold breaking points. *J Appl Physiol* 37: 291–296, 1974.

2. **Cross TJ**, **Breskovic T**, **Sabapathy S**, **Maslov PZ**, **Johnson BD**, **Dujic Z**. Respiratory muscle pressure development during breath holding in apnea divers. *Med Sci Sports xerc* 45: 93–101, 2013.

3. **Bellemare F**, **Grassino A**. Effect of pressure and timing of contraction on human diaphragm fatigue. *J Appl Physiol* 53: 1190–1195, 1982.

4. **Zocchi L**, **Fitting JW**, **Majani U**, **Fracchia C**, **Rampulla C**, **Grassino A**. Effect of pressure and timing of contraction on human rib cage muscle fatigue. *Am Rev Respir Dis* 147: 857–864, 1993.

5. **Graham BL**, **Steenbruggen I**, **Miller MR**, **Barjaktarevic IZ**, **Cooper BG**, **Hall GL**, **Hallstrand TS**, **Kaminsky DA**, **McCarthy K**, **McCormack MC**, **Oropez CE**, **Rosenfeld M**, **Stanojevic S**, **Swanney MP**, **Thompson BR**. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. *Am J Respir Crit Care Med* 200: e70–e88, 2019.

6. **Quanjer PH**, **Stanojevic S**, **Cole TJ**, **Baur X**, **Hall GL**, **Culver BH**, **Enright PL**, **Hankinson JL**, **Ip MSM**, **Zheng J**, **Stocks J**, **Initiative EGLF**. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 40: 1324–1343, 2012.

7. **Peters CM**, **Welch JF**, **Dominelli PB**, **Molgat-Seon Y**, **Romer LM**, **McKenzie DC**, **Sheel AW**. Influence of inspiratory resistive loading on expiratory muscle fatigue in healthy humans. *Exp Physiol* 102: 1221–1233, 2017.

8. **Welch JF**, **Archiza B**, **Guenette JA**, **West CR**, **Sheel AW**. Effect of diaphragm fatigue on subsequent exercise tolerance in healthy men and women. *J Appl Physiol* 125: 1987–1996, 2018.

9. **Taylor BJ**, **Romer LM**. Effect of expiratory resistive loading on inspiratory and expiratory muscle fatigue. *Respir Physiol Neurobiol* 166: 164–174, 2009.

10. **Laurie SS**, **Yang X**, **Elliott JE**, **Beasley KM**, **Lovering AT**. Hypoxia-induced intrapulmonary arteriovenous shunting at rest in healthy humans. *J Appl Physiol* 109: 1072–1079, 2010

11. **Laveneziana P**, **Albuquerque A**, **Aliverti A**, **Babb T**, **Barreiro E**, **Dres M**, **Dubé B-P**, **Fauroux B**, **Gea J**, **Guenette JA**, **Hudson AL**, **Kabitz H-J**, **Laghi F**, **Langer D**, **Luo Y-M**, **Neder JA**, **O'Donnell D**, **Polkey MI**, **Rabinovich RA**, **Rossi A**, **Series F**, **Similowski T**, **Spengler C**, **Vogiatzis I**, **Verges S**. ERS statement on respiratory muscle testing at rest and during exercise. *Eur Respir J* 53: 1801214, 2019.

12. **Whitelaw WA**, **McBride B**, **Amar J**, **Corbet K**. Respiratory neuromuscular output during breath holding. *J Appl Physiol* 50: 435–443, 1981.

13. **Agostoni E**. Diaphragm activity during breath holding: factors related to its onset. *J Appl Physiol* 18: 30–36, 1963.

14. **Sempik AK**. An electromyographic study of intercostal muscle activity in man during breath-holding [proceedings]. *J Physiol* 276: 10P-11P, 1978.

15. **Sempik A**, **Patrick JM**. The effect of thoracic vibration on ventilation and breath-holding in man. *Respir Physiol* 44: 381–391, 1981.

16. **Kulik AM**. Electrical activity of the respiratory muscles in man during breath holding. *Bull Exp Biol Med* 57: 402–407, 1964.

17. **Yan S**, **Lichros I**, **Zakynthinos S**, **Macklem PT**. Effect of diaphragmatic fatigue on control of respiratory muscles and ventilation during CO_2 rebreathing. *J Appl Physiol* 75: 1364–1370, 1993.

18. **Laghi F**, **Topeli A**, **Tobin MJ**. Does resistive loading decrease diaphragmatic contractility before task failure? *J Appl Physiol* 85: 1103–1112, 1998.

19. **Roussos C**, **Fixley M**, **Gross D**, **Macklem PT**. Fatigue of inspiratory muscles and their synergic behavior. *J Appl Physiol* 46: 897–904, 1979.

20. **Levitzky MG**. *Pulmonary Physiology*. 8th ed. McGraw Hill Education, 2013.

21. **Willie CK**, **Ainslie PN**, **Drvis I**, **MacLeod DB**, **Bain AR**, **Madden D**, **Maslov PZ**, **Dujic Z**. Regulation of brain blood flow and oxygen delivery in elite breath-hold divers. *J Cereb Blood Flow Metab* 35: 66–73, 2014.

22. **Babcock MA**, **Johnson BD**, **Pegelow DF**, **Suman OE**, **Griffin D**, **Dempsey JA**. Hypoxic effects on exercise-induced diaphragmatic fatigue in normal healthy humans. *J Appl Physiol* 78: 82–92, 1995.

23. **Vogiatzis I**, **Georgiadou O**, **Giannopoulou I**, **Koskolou M**, **Zakynthinos S**, **Kostikas K**, **Kosmas E**, **Wagner H**, **Peraki E**, **Koutsoukou A**, **Koulouris N**, **Wagner PD**, **Roussos C**. Effects of exercise-induced arterial hypoxaemia and work rate on diaphragmatic fatigue in highly trained endurance athletes. *J Physiol* 572: 539–549, 2006.

24. **Vogiatzis I**, **Georgiadou O**, **Koskolou M**, **Athanasopoulos D**, **Kostikas K**, **Golemati S**, **Wagner H**, **Roussos C**, **Wagner PD**, **Zakynthinos S**. Effects of hypoxia on diaphragmatic fatigue in highly trained athletes. *J Physiol* 581: 299–308, 2007.

25. **Vogiatzis I**, **Athanasopoulos D**, **Boushel R**, **Guenette JA**, **Koskolou M**, **Vasilopoulou M**, **Wagner H**, **Roussos C**, **Wagner PD**, **Zakynthinos S**. Contribution of respiratory muscle blood flow to exercise-induced diaphragmatic fatigue in trained cyclists. *J Physiol* 586: 5575–5587, 2008.

26. **Rafferty GF**, **Harris ML**, **Polkey MI**, **Greenough A**, **Moxham J**. Effect of hypercapnia on maximal voluntary ventilation and diaphragm fatigue in normal humans. *Am J Respir Crit Care Med* 160: 1567–1571, 1999.

27. **Carroll TJ**, **Taylor JL**, **Gandevia SC**. Recovery of central and peripheral neuromuscular fatigue after exercise. *J Appl Physiol* 122: 1068–1076, 2017.

FIGURE LEGENDS

Figure 1. Experimental protocol overview. This is a schematical representation of the experimental protocol.

Figure 2. Apnea durations. Box plots of all preparatory apneas (white) and maximal (gray) apneas are expressed as seconds.

Figure 3. Final apnea data tracings. This is a tracing of the diaphragm EMG, arterial oxygen saturation (SpO_2) and heart rate (HR) during the final maximal apnea. Note the stark fall in SpO_2 at the onset of the struggle phase, i.e., IBMs begin. Additionally, note that diaphragm EMG activity is minimal, but that there are “bursts” of activity during the struggle phase which is a reflection of diaphragm contraction.

Figure 4. Maximal inspiratory and expiratory pressures. Maximal inspiratory pressure generating ability (MIP) is displayed in panel A. Maximal expiratory pressure generating ability (MEP) is displayed in panel B. There was no change in MIP or MEP following the apnea protocol.

Additional Information Section:

Competing Interests: The authors have no conflicts of interest.

Author contributions: JWD and CDH designed the study. ID designed the apnea protocol. DV and NF enrolled participants. All authors contributed to data collection and analyses. JWD drafted the manuscript. All authors had complete access to all of the study data and critically revised the manuscript. All authors approved the final version of the manuscript and take responsibility for the integrity of the data and accuracy of the data analysis.

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Data Availability: The data will be made available upon reasonable request to the corresponding author.

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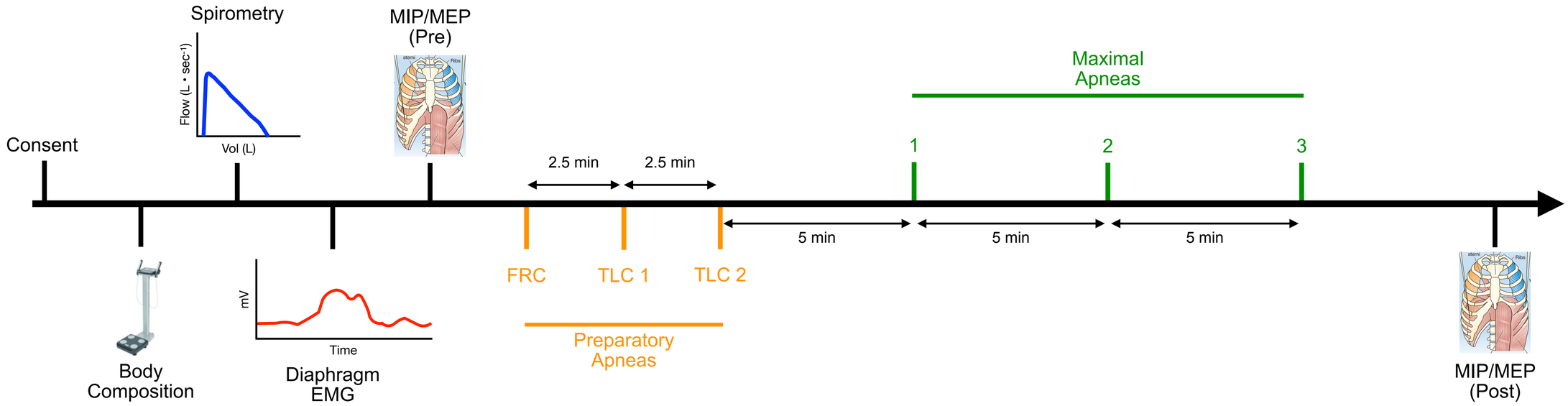
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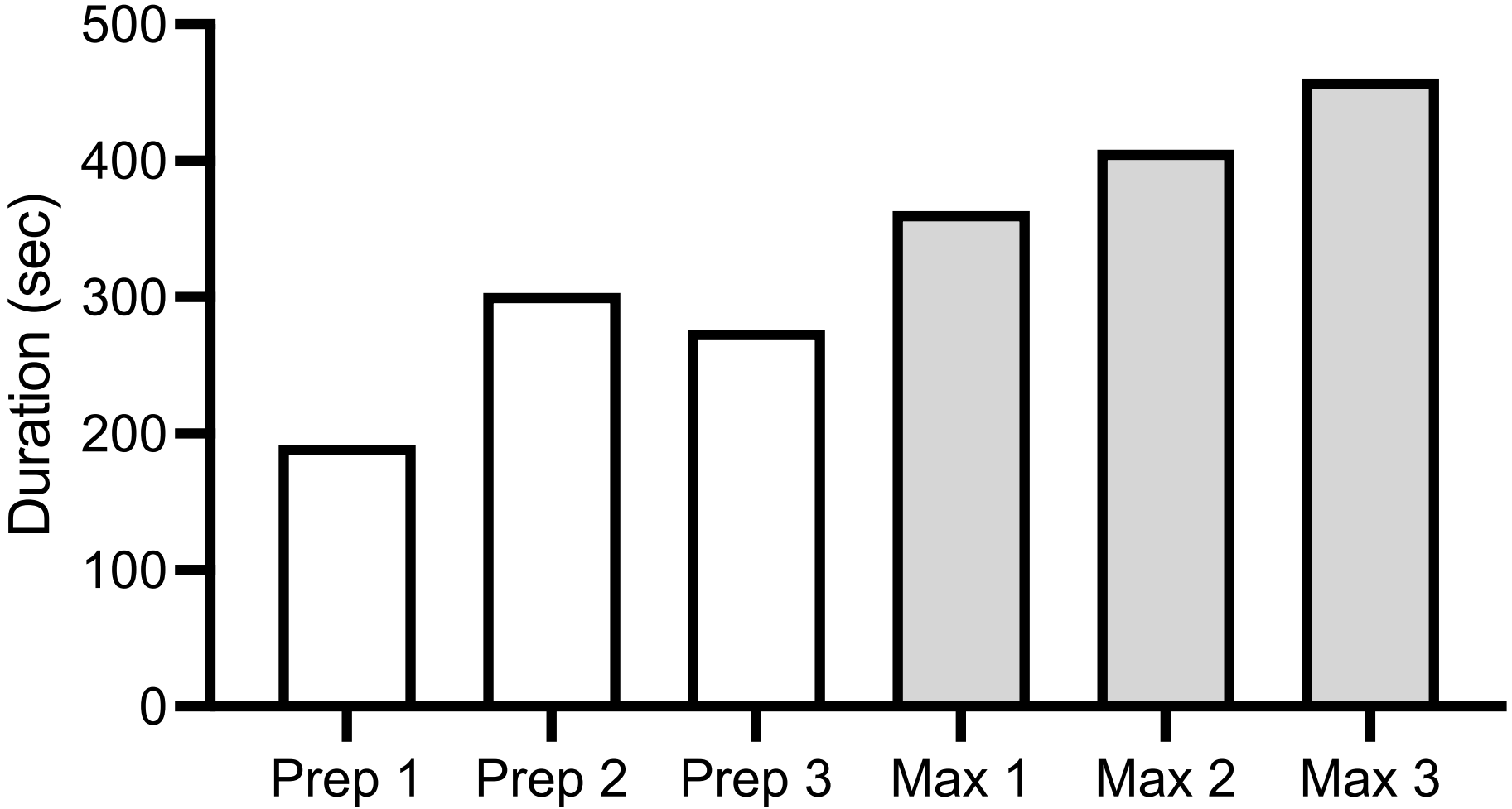
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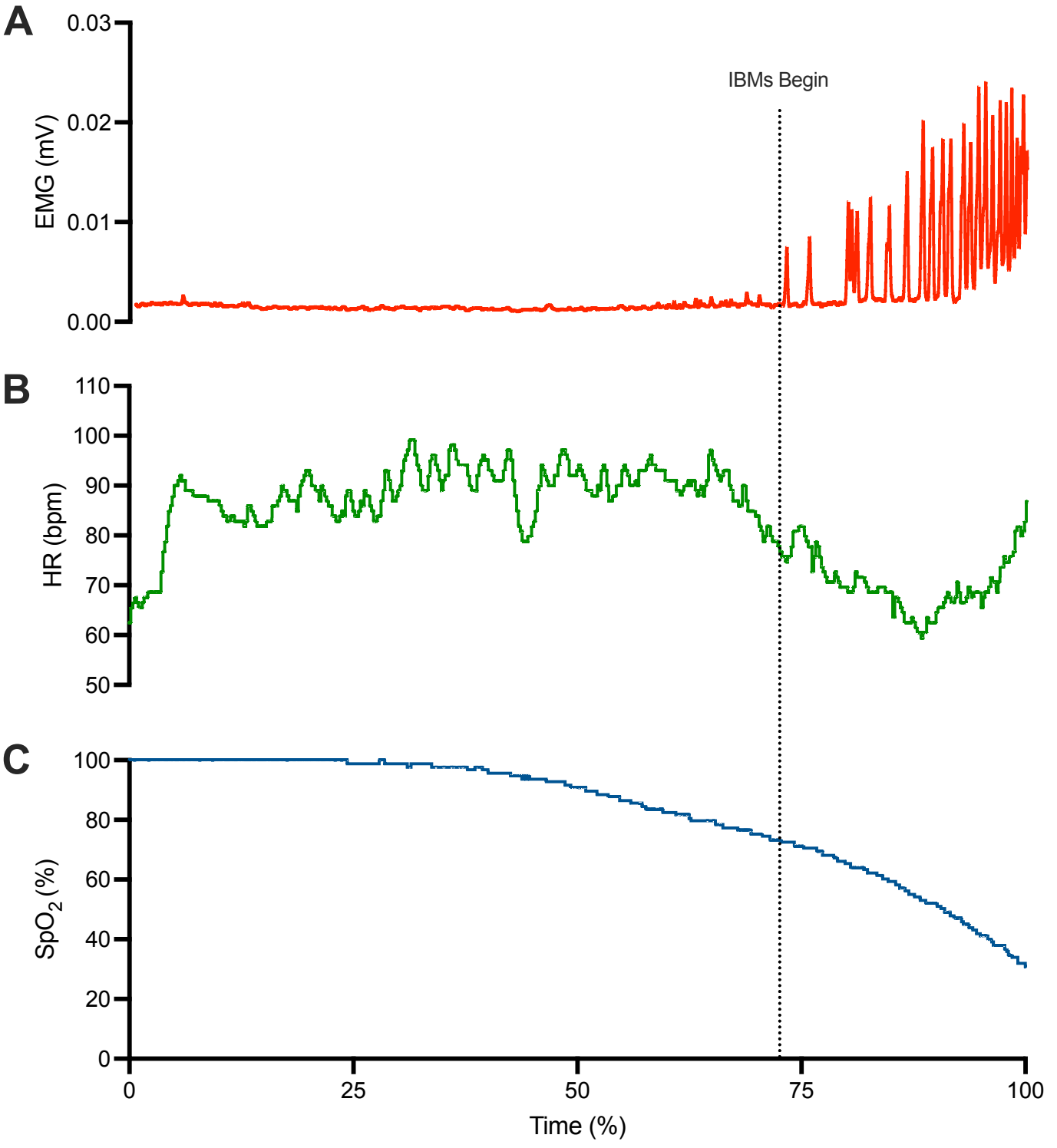
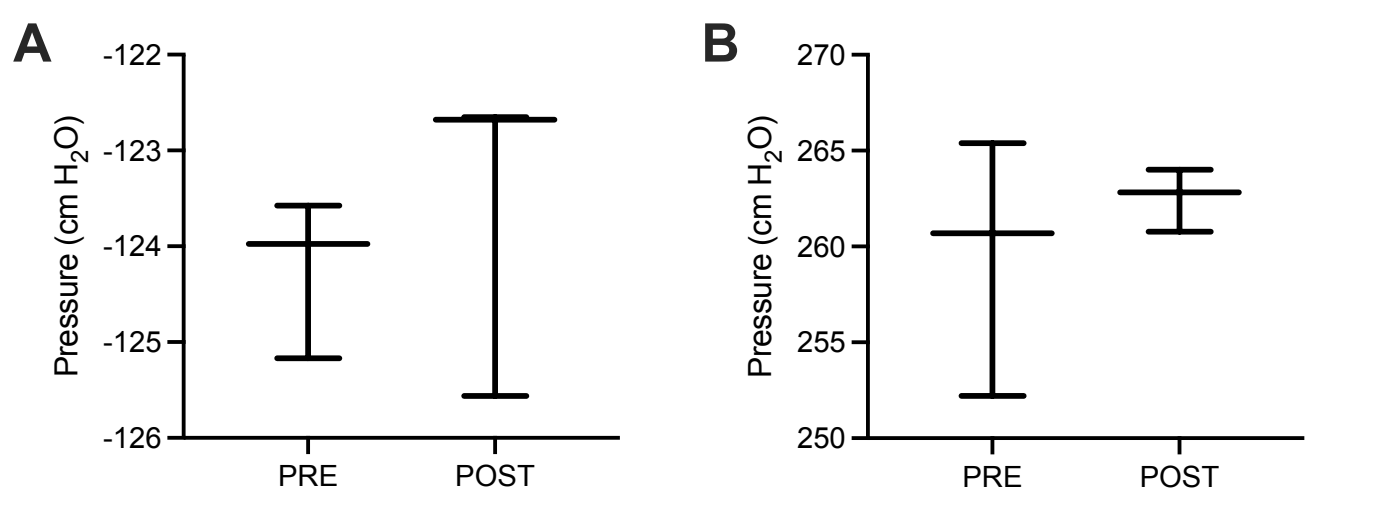
Max 2
Max 3


A
0.03
0.02
0.01
0.00
EMG (mV)
IBMs Begin
B
110
100
90
80
70
60
50
HR (bpm)
C
100
80
60
40
20
0
SpO2 (%)
0
25
50
75
100
Time (%)


A
-122
-123
-124
-125
-126
Pressure (cm H2O)
PRE
POST
B
270
265
260
255
250
Pressure (cm H2O)
PRE
POST