



ELSEVIER

Contents lists available at ScienceDirect

MethodsX

journal homepage: [www.elsevier.com/locate/mex](http://www.elsevier.com/locate/mex)



## Protocol Article

# PEA: Five maximum repeated apnea maneuvers prior to middle-distance racing



Dimitrios I. Bourdas

Section of Sport Medicine & Biology of Exercise, School Physical Education and Sport Science, National and Kapodistrian University of Athens, 41 Ethnikis Antistasis, 17237 Daphne, Greece

## ABSTRACT

It was hypothesized that executing repeated maximum apnea efforts would improve performance in a subsequent time to exhaustion test. Indeed, in young moderately fit male subjects without former experience in apnea has been shown that five repeated apnea maximal efforts with face immersion in cold water (PEA) have advantageous effect to consecutive performance in a time to exhaustion ride without being further affected by apnea training of two weeks. So, in the current article, we describe, in details, the protocol procedure and the technical steps of the five maximum-repeated apnea maneuvers prior to a middle-distance racing in order to improve performance, from our previous relevant published research.

© 2021 The Author(s). Published by Elsevier B.V.  
This is an open access article under the CC BY-NC-ND license  
(<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

## ARTICLE INFO

Protocol name: Pre Exercise Apneas (PEA)

Keywords: Priming apnea, Exercise preconditioning, Hypercapnia, Novice divers, Performance, Reliability, Breath hold

Article history: Received 1 July 2021; Accepted 12 August 2021; Available online 13 August 2021

## Specifications table

Subject Area:	Medicine and Dentistry
More specific subject area:	Exercise Physiology and Performance
Protocol name:	Pre Exercise Apneas (PEA)

(continued on next page)

DOI of original article: [10.1016/j.resp.2021.103703](https://doi.org/10.1016/j.resp.2021.103703)

E-mail addresses: [dbourdas@phed.uoa.gr](mailto:dbourdas@phed.uoa.gr), [dbourdas@hotmail.com](mailto:dbourdas@hotmail.com)

<https://doi.org/10.1016/j.mex.2021.101490>

2215-0161/© 2021 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license  
(<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

---

Reagents/tools:	<i>Weight &amp; height scale: Bilance, SALUS, Milano, Italy</i> <i>Metabolic stress testing system: CPX/Ultima, MedGraphics, Saint Paul, USA</i> <i>Cycle Ergometer: Corival CPET, Lode BV, Groningen, The Netherlands</i> <i>Noseclip: CS05, Paradisia, Villefranche-sur-mer, France</i> <i>Respiratory valve: 3 Way Valve # 2870, Hans Rudolph, Inc., Kansas City, USA</i> <i>Respiratory valve: 3 Way Stopcock # 2100, Hans Rudolph, Inc., Kansas City, USA</i> <i>Breathing Bag: #1193-5, Vacumed, Ventura, USA</i> <i>3 Liter Calibration Syringe: Series 704001-004, MedGraphics, Saint Paul, USA</i> <i>Stopwatch: Casio HS30W, Casio Computer CO., LTD. Tokyo, Japan</i> <i>Oximeter: NELLCOR Symphony N-3000, Avante, Charlotte, USA</i>
Experimental design:	<i>Two min prior to a timed ride to exhaustion at 150% of peak power output achieved with an exhaustive maximal oxygen uptake effort, participant completes a set of five repeated apnea maximal efforts with face immersion in cold water, separated by 2-min recovery intervals.</i>
Trial registration:	<i>Not applicable</i>
Ethics:	<i>Ethical approval for this procedure was granted by the School of Physical Education and Sport Science / National and Kapodistrian University of Athens bio-ethics committee</i>
Value of the Protocol:	<ul style="list-style-type: none"> <li>• <i>PEA induced favorable hematological changes</i></li> <li>• <i>PEA enhanced contribution of aerobic metabolism in subsequent high-intensity exercise performance</i></li> <li>• <i>PEA serves as a tool to improve (~11%) middle-distance racing performance</i></li> </ul> <i>[D.I. Bourdas, N.D. Geladas, Five repeated maximal efforts of apneas increase the time to exhaustion in subsequent high-intensity exercise, Respir. Physiol. Neurobiol. 292 (2021) 103703. <a href="https://doi.org/10.1016/j.resp.2021.103703">https://doi.org/10.1016/j.resp.2021.103703</a>.]</i>

---

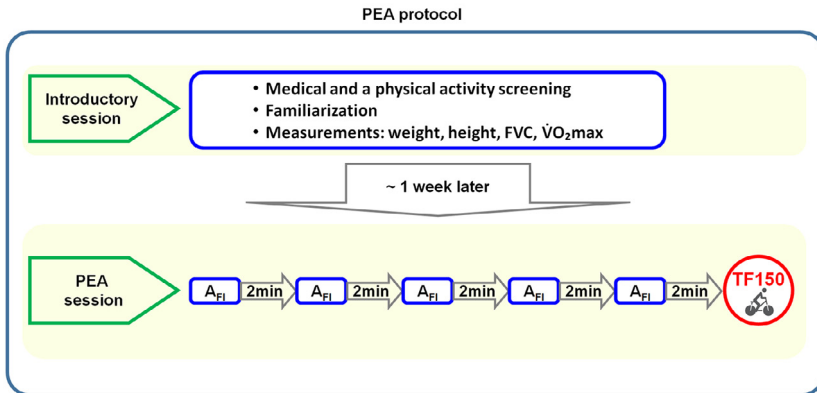
## Background

Apneas (or breath holds) in a repetitive manner induce – favorable hematological and blood buffering changes [1–4] and for that reason, it had been hypothesized that repeated apnea maximal efforts may subsequently enhance performance in a time to exhaustion test [5,6]. In 2011, at the XIV International Conference On Environmental Ergonomics, the first empirical data were presented, as far as we know, which were showed that five repeated apneas with face immersion in cold water increase the time to exhaustion at 150% of peak power output [6]. Few years later, a research paper originally published by D. Bourdas and N. Geladas in 2021, presented data derived from novice to apnea male subjects which revealing, that five repeated apneas maximal efforts with face immersion in cold water improve oxygen delivery to skeletal muscles and increase time to exhaustion (11.3%) in subsequent high-intensity exercise of short-duration (i.e., using the same experimental design us earlier) without being further affected (11.7%) by apnea training of two weeks [7].

## Method details

### *Experimental procedures*

In this section, technical information for the Pre Exercise Apneas (PEA) protocol procedure is presented. This procedure requires the participant tested  $\tau_0$  visit the laboratory on two separate days, corresponding to introductory and PEA session (Fig. 1). Inclusion criteria for participation are: unfamiliarity with apnea activities, age  $\geq 18$  yr, at least moderate physical activity ( $> 1000$  MET-min $\cdot$ week $^{-1}$ ), good general health and freedom from any kind of medication. In the introductory session, participant firstly completes a pre-study medical and a physical activity questionnaire [8–10], is informed of any potential risks that might derive from the experimental procedure and signs a consent form for participation in the study, in accordance with the Helsinki Declaration [11]. Next, participant being familiarized with the laboratory equipment and methods, is educated to perform apneas in as a relaxed, focused and concentrated manner as possible. It is also emphasized to the participant that respiratory manipulations (i.e., hyperventilating, swallowing, or exhaling or performing any kind of maneuvers like Valsava and Mueller) are not allowed before apneas. The introductory session ends with the conduction of all necessary physiological measurements such as weight, height (Bilance, SALUS, Milano, Italy), force vital capacity (FVC) in a sitting position



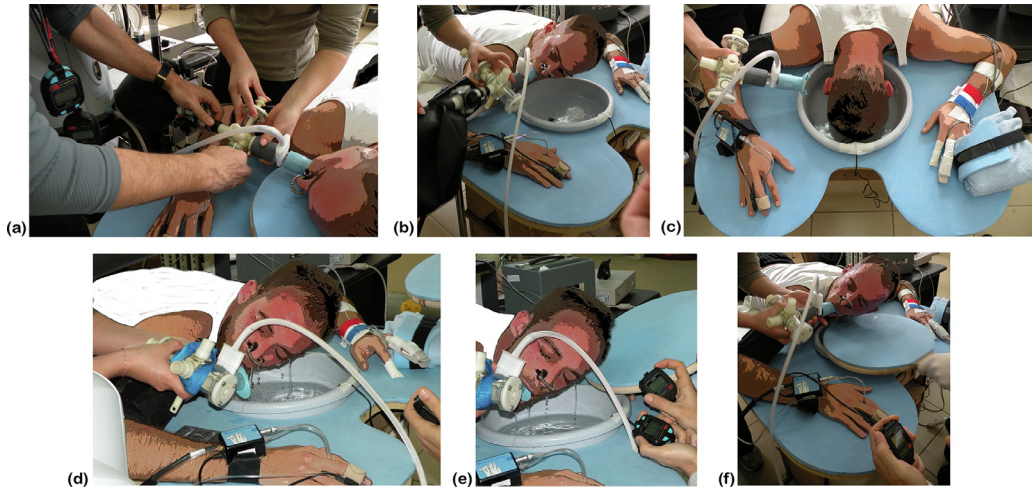
**Fig. 1.** Overview of the five repeated apneas maximal efforts with face immersion in cold water protocol prior to a time to exhaustion test. Abbreviations: A<sub>FI</sub>, maximum breath hold with face immersion in cold water; FVC, force vital capacity; PEA, pre exercise apneas; TF150, cycling test to exhaustion at intensity corresponding to 150% of the peak power output; VO<sub>2</sub>max, maximum oxygen uptake; 2min, two minutes rest.



**Fig. 2.** On a recliner (a), the participant tested in prone position lying down with his upper limbs in a position of ascension while just below his head there is a covered water tank with water 5l at a constant temperature at 12°C (b). (c) The participant tested during apnea by immersion of his face in the water tank.

(CPX/Ultima, MedGraphics, Saint Paul, USA), and maximum oxygen uptake (VO<sub>2</sub>max) during a graded exercise test on a cycle ergometer (CPX/Ultima, MedGraphics, Saint Paul, USA; Corival CPET, Lode BV, Groningen, The Netherlands), according to established guidelines and criteria for exercise testing [12].

Few days later (~ one week), in the PEA session, prior to exercise test the participant perform PEA (i.e., set of five repeated maximal-effort apneas, separated by 2-min recovery intervals, with face immersion in cold water (12°C), in prone position with her/his arms always beside her/his head at the same level; Fig. 2) [7,13,14]. Underneath the participant's head is placed the water container (6.5 l). The container is covered by a removable soft surface so allowing head rest and relax for several minutes (~7 min) until resting heart rate (HR) being stabilized (Fig. 2). The participant has to wear a nose clip (Noseclip CS05, Paradisia, Villefranche-sur-mer, France) and breath via a mouthpiece with one manual three-way valve (3 Way Valve # 2870, Hans Rudolph, Inc., Kansas City, USA). The valve is connected in a series to another valve (3 Way Stopcock # 2100, Hans Rudolph, Inc., Kansas City, USA), which is attached to a 5 l maximum capacity elastic vinyl bag (#1193-5 Breathing Bag, Vacumed, Ventura, USA), filled (3 Liter Calibration Syringe-Series 704001-004, MedGraphics, Saint Paul, USA) with 85% of individual's FVC atmospheric air, from which participant inhaled prior to each apnea effort. Participant is informed verbally (by a stand by researcher) at 60, 30, and 15 sec prior to each apnea. Participant is also reminded to elevate her/his head as the water container cover is removed with a final signal at 10 sec prior to each apnea. At that point, participant fully exhales to hers/his residual volume and inhales the atmospheric air from the connected vinyl bag. After that,



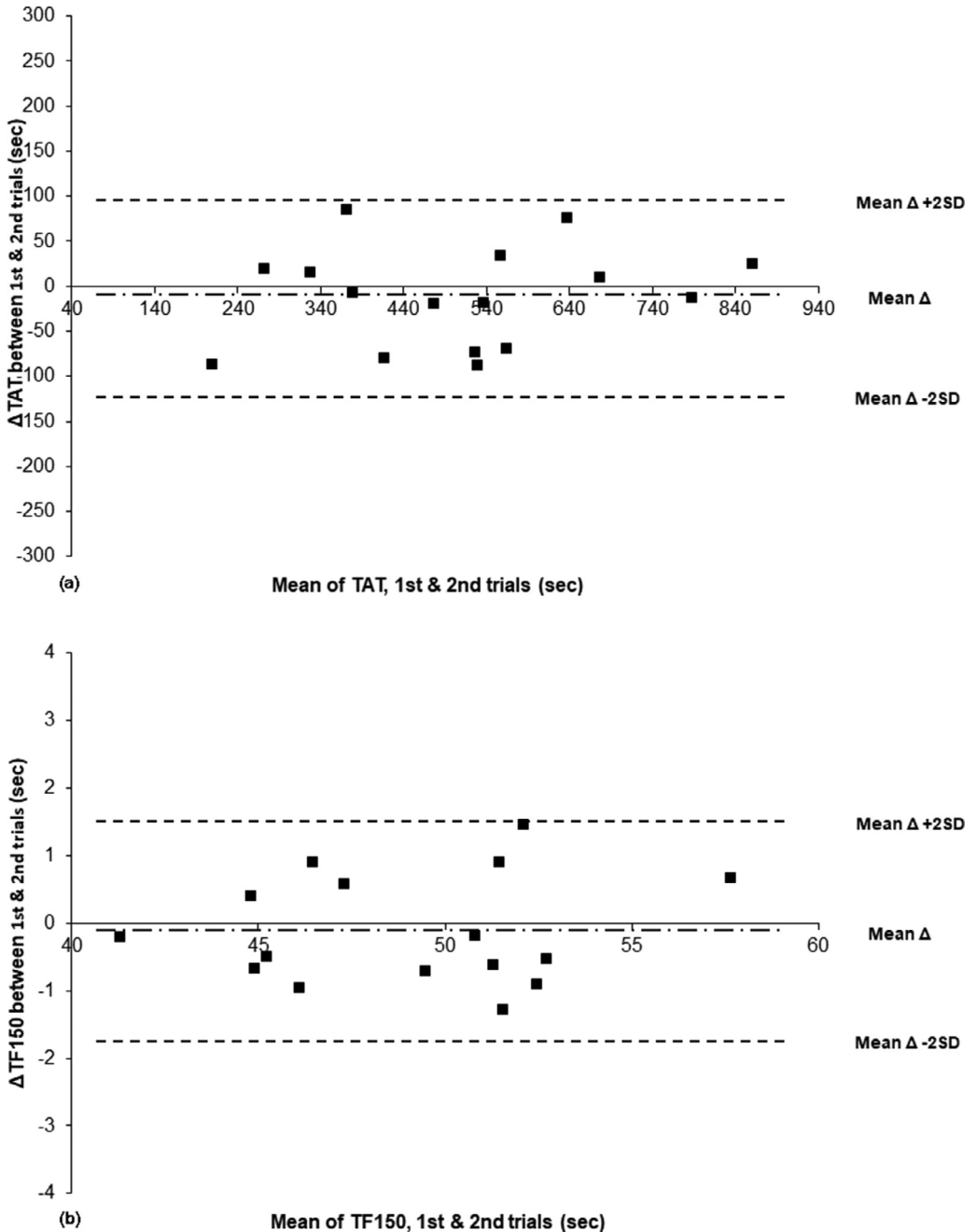
**Fig. 3.** The researchers connect the participant with a vinyl bag filled with atmospheric air at 85% of its force vital capacity through a series of a mouthpiece and two valves (a). The participant, inhales the bag's atmospheric air and holds his breath (b), immerses his face into the water tank (c), raises his head from the water tank and turns it to the right (d), accepts the mouthpiece (e) and exhales into the mouthpiece (f), just before calming down after the end of apnea.

a researcher removes the mouthpiece which is connected with the vinyl bag, and the participant instantly immerses her/his face in the cold water, including forehead and chin. Upon cessation of each apnea maneuver, participant raises her/his face above the water surface, tilted the head to the right, and a researcher inserts the mouthpiece into the mouth in the proper way for exhalation while the container's soft cover is repositioned for head resting (Fig. 3).

Two minutes after the last apnea effort, participant performs a timed to exhaustion exercise ride, such as the TF150 [15], on an electronically braked ergometer (Corival CPET, Lode BV). The TF150 exercise test requires warm-up at  $1 \text{ Watt} \cdot \text{kg}^{-1}$  (body mass) load for 150 sec at 50–75 rpm (ad libitum) of which the last 15 sec, participant increases cycling speed to 90 rpm. In the next 5 sec, participant increases speed to its maximum potential and while the intensity (load) is increased (by a researcher at the 150% of the peak power output achieved during  $\dot{V}O_2\text{max}$  measurement) continually rides at maximum frequency until exhaustion. As TF150 performance considered the time (Casio HS30W, Casio Computer CO., LTD. Tokyo, Japan) from when the participant started cycling with maximum speed to the point at which she/he could not maintain a riding frequency  $\geq 70$  rpm. Moreover, for safety reasons, monitoring of peripheral capillary oxygen saturation and HR by a pulse oximeter probe (NELCOR Symphony N-3000, Avante, Charlotte, USA) is strongly advisable. Optional, other physiological measurements can be added to the existing procedure (e.g., respiratory gases, hemodynamics, skin blood flow, blood gases, electrocardiogram, etc.; Fig. 3). It is also understood that prior to each session (introductory and PEA), all laboratory instruments must be calibrated according to the manufacturers' manuals.

### Reliability

The PEA + TF150 reliability were tested twice (using the aforementioned procedure) in 16 adult male subjects (age:  $21.3 \pm 2.2$  yr (20.2 – 22.3), height:  $180.3 \pm 5.7$  cm (177.5 – 183.1), body mass:  $83.2 \pm 10.1$  kg (78.2 – 88.2); data presented as mean  $\pm$  standard deviation (95% confidence interval)). The two series of PEA prior to TF150 was conducted with an interval of one week. All volunteers arrived in the lab having done no intense activity in the previous three days and were instructed to have the same meals in order to replicate the pretrial macronutrient consumption as closely as possible. The laboratory environment remained consistent throughout the investigation (24–26°C air temperature



**Fig. 4.** PEA reliability evaluation. Difference between values of a first and a second trial of TAT against their mean in sixteen subjects (a). Difference between values of a first and a second trial of TF150 exercise performance against their mean in sixteen subjects (b). The mean  $\Delta \pm SD$  is  $-12.77 \pm 55.25$  sec for TAT and  $-0.12 \pm 0.81$  sec for TF150, and with a 95% confidence interval; the limits of agreement ( $97.73$  sec to  $-123.28$  sec =  $\pm 2$  SD and  $1.51$  sec to  $-1.74$  sec =  $\pm 2$  SD, respectively) are small enough to consider the PEA reliable. Abbreviations:  $\Delta$ , of two trials; PEA, pre exercise apneas; SD, standard deviation; TAT, sum of five apneas time; TF150, cycling test to exhaustion at intensity corresponding to 150% of the peak power output.

and  $52 \pm 5\%$  relative humidity). Until the end of the trials, subjects received no feedback on their breath hold time or TF150 performance and no information was given that could lead to favorable or negative expectations about the project's conclusion. Moreover, the real purpose of the study was unknown to both the subjects and the researchers.

A high Pearson correlation coefficient (PCC) between the two repeated trials of total apnea time (TAT: sum of the five apneas time) was provided ( $r = 0.95$ ,  $P \leq 0.001$ ). The coefficient of variation (CV) of mean difference ( $\Delta$ ) values between the two trials of TAT measurements was 10.8%. In addition, a high PCC between the two repeated trials of TF150 post PEA was also provided ( $r = 0.98$ ,  $P \leq 0.001$ ). The CV of mean  $\Delta$  values between the two trials of measurements of TF150 was 1.7%. The mean  $\Delta \pm$  standard deviation between the two trials were at an acceptable level of  $-12.77 \pm 55.25$  sec and  $-0.12 \pm 0.81$  sec for TAT and TF150 (respectively), and with a 95% confidence interval according to the Bland–Altman plot [16]; the limits of agreement (97.73 sec to  $-123.28$  sec and 1.51 sec to  $-1.74$  sec, respectively) were small enough to consider the procedure reliable, (Fig. 4).

### Perspective

It seems that PEA is a very promising protocol in order to improve middle-distance racing performance and that may play an important role in improving the time performance when high intensity exercise follows on [7]. However, PEA protocol has been studied only in young moderately physically active men without former experience in apnea and in one type, mode, and duration of exercise, which limits its generalizability. Therefore further research is needed to apply PEA in various competitive exercise settings and competitive athletes.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### References

- [1] D. Baković, D. Eterović, Ž. Saratlija-Novaković, I. Palada, Z. Valic, N. Bilopavlović, Ž. Dujić, Effect of human splenic contraction on variation in circulating blood cell counts, *Clin. Exp. Pharmacol. Physiol.* 32 (2005) 944–951, doi:[10.1111/j.1440-1681.2005.04289.x](https://doi.org/10.1111/j.1440-1681.2005.04289.x).
- [2] D. Baković, Z. Valic, D. Eterović, I. Vuković, A. Obad, I. Marinović-Terzić, Ž. Dujić, Spleen volume and blood flow response to repeated breath-hold apneas, *J. Appl. Physiol.* 95 (2003) 1460–1466, doi:[10.1152/jappphysiol.00221.2003](https://doi.org/10.1152/jappphysiol.00221.2003).
- [3] F. Joulia, J.G. Steinberg, M. Faucher, T. Jamin, C. Ulmer, N. Kipson, Y. Jammes, Breath-hold training of humans reduces oxidative stress and blood acidosis after static and dynamic apnea, *Respir. Physiol. Neurobiol.* 137 (2003) 19–27, doi:[10.1016/S1569-9048\(03\)00110-1](https://doi.org/10.1016/S1569-9048(03)00110-1).
- [4] E. Schagatay, H. Haughey, J. Reimers, Speed of spleen volume changes evoked by serial apneas, *Eur. J. Appl. Physiol.* 93 (2005) 447–452, doi:[10.1007/s00421-004-1224-0](https://doi.org/10.1007/s00421-004-1224-0).
- [5] F. Lemaître, F. Joulia, D. Chollet, Apnea: a new training method in sport? *Med. Hypotheses.* 74 (2010) 413–415, doi:[10.1016/j.mehy.2009.09.051](https://doi.org/10.1016/j.mehy.2009.09.051).
- [6] D. Bourdas, A. Kostantopoulos, T. Tsakiris, K. Pavlakis, D. Triantafyllou, N. Geladas, Repeated breath holds increase the time to exhaustion at 150% of peak power output, in: S. Kounalakis, M. Koskolou (Eds.), 14th Int. Conf. Environ. Ergon. 2011, National and Kapodestrian University of Athens, Nafplion Greece, 2011, pp. 233–235.
- [7] D.I. Bourdas, N.D. Geladas, Five repeated maximal efforts of apneas increase the time to exhaustion in subsequent high-intensity exercise, *Respir. Physiol. Neurobiol.* 292 (2021) 103703, doi:[10.1016/j.resp.2021.103703](https://doi.org/10.1016/j.resp.2021.103703).
- [8] D. Warburton, V. Jamnik, S. Bredin, N. Gledhill, The 2018 physical activity readiness questionnaire for everyone (PAR-Q+) and electronic physical activity readiness medical examination (ePARmed-X+), *Heal. Fit. J. Canada.* 11 (2018) 31–34, doi:[10.14288/hfjc.v11i1.260](https://doi.org/10.14288/hfjc.v11i1.260).
- [9] D.I. Bourdas, E.D. Zacharakis, Impact of COVID-19 lockdown on physical activity in a sample of Greek adults, *Sports* 8 (2020) 139, doi:[10.3390/sports8100139](https://doi.org/10.3390/sports8100139).
- [10] D.I. Bourdas, E.D. Zacharakis, Evolution of changes in physical activity over lockdown time: physical activity datasets of four independent adult sample groups corresponding to each of the last four of the six COVID-19 lockdown weeks in Greece, *Data Br.* 32 (2020) 106301, doi:[10.1016/j.dib.2020.106301](https://doi.org/10.1016/j.dib.2020.106301).
- [11] World Medical Association, Declaration of Helsinki, ethical principles for scientific requirements and research protocols, *Bull. World Health Organ.* 79 (2013) 373 <https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/>.
- [12] American College of Sports Medicine/ACSM's Guidelines For Exercise Testing And Prescription, 10th ed., Wolters Kluwer Health, Philadelphia, PA, 2018.
- [13] D.I. Bourdas, T.S. Tsakiris, K.I. Pavlakis, D.V. Triantafyllou, N.D. Geladas, Repeated apneas and hypercapnic ventilatory response before and after apnea training, *Aerosp. Med. Hum. Perform.* 86 (2015) 27–33, doi:[10.3357/AMHP.3932.2015](https://doi.org/10.3357/AMHP.3932.2015).

- [14] D.I. Bourdas, T.S. Tsakiris, A.I. Konstantopoulos, D.V. Triantafillou, N.D. Geladas, Hypercapnic ventilatory response: a comparison between elite and novice skin divers, *Open Sport. Med. J.* 8 (2014) 16–22, doi:[10.2174/1874387001408010016](https://doi.org/10.2174/1874387001408010016).
- [15] F.H. Lindsay, J.A. Hawley, K.H. Myburgh, H.H. Schomer, T.D. Noakes, S.C. Dennis, Improved athletic performance in highly trained cyclists after interval training, *Med. Sci. Sport. Exerc.* 28 (1996) 1427–1434, doi:[10.1097/00005768-199611000-00013](https://doi.org/10.1097/00005768-199611000-00013).
- [16] J.M. Bland, D.G. Altman, Statistical methods for assessing agreement between two methods of clinical measurement, *Lancet* 327 (1986) 307–310, doi:[10.1016/S0140-6736\(86\)90837-8](https://doi.org/10.1016/S0140-6736(86)90837-8).