



# Effects of Five Serial Apnoeas Prior to a Running Time Trial in Competitive Athletes

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## Abstract

**Purpose** This study aimed to determine whether a series of repeated maximal voluntary apnoeas is effective in improving subsequent time trial performance in competitive level track and field athletes.

**Methods** Seventeen competitive runners volunteered for this study and based on their preferred competitive distance they were placed either in the 200 m (5 male, 4 female) or 1000 m group (3 male, 5 female). On two separate occasions ( $\leq 7$  days apart), the participants performed a running time-trial that was preceded either by: (i) a standardised warm up (WO) or (ii) a standardised warm up succeeded by five repeated maximal dry static apnoeas (WA). Splenic volume, haematology and cardiovascular parameters were monitored at rest, before and after each time-trial.

**Results** WA resulted in a significantly faster performance ( $27.51 \pm 3.49$  s;  $P=0.009$ ) compared with WO ( $27.96 \pm 3.34$  s) in the 200 m group, whereas no differences were observed in the 1000 m group (WA,  $211.10 \pm 26.18$  s; WO,  $215.82 \pm 25.13$  s,  $P=0.120$ ). No differences were noted in splenic volume between WO and WA in either group ( $P \geq 0.081$ ). Haemoglobin was significantly elevated after breath-holding in the 200 m ( $+7$  g/dL,  $P=0.041$ ) but not 1000 m group.

**Conclusion** This study demonstrates that five repeated maximal apnoeas are capable of significantly improving a 200 m but not a 1000 m time-trial performance in competitive track and field athletes.

## Introduction

In elite athletics, marginal gains are a key priority for athletes and their coaches [10]. Evidence suggests that apnoea (breath-holding) might provide a method for improving performance in various activities either as a supplementary training method [19] or as a priming activity prior to

exercise [16]. Acute apnoea causes a number of physiological responses that have an oxygen conserving effect within the body [7]. Collectively these responses are known as the mammalian diving reflex and include bradycardia [1], vasoconstriction of selected vascular beds [15] reduced blood flow to peripheral capillary beds [9], increased sympathetic outflow to the periphery and splenic contraction [12].

The spleen is the largest lymphoid organ in the human body and roughly contains 200–250 mL of blood [22]. During exercise and breath-holding, where the sympathetic tone is elevated, the spleen contracts, consequently releasing its stored erythrocytes into the systemic circulation [14]. Notably, haemoglobin increases of up to 8 g/dL have previously been recorded in non-apnoea trained cohorts following a series of 3–5 maximal static apnoeas, with these reported to persist up to 10 min after the last apnoeic bout. Albeit transient, these increases could theoretically improve the oxygen carrying capacity of blood and the carbon dioxide

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clearance rate, both considered advantageous from a performance perspective. Accordingly, in the past decade several studies have explored the potential utility of breath-holding as a priming strategy prior to endurance events, with these having yielded equivocal results.

Sperlich et al. [21] was the first to investigate the effects of pre-exercise breath-holding on a 4-km cycling time-trial performance. Although splenic contractions were noted after the apnoeic intervention, these were not sufficient to invoke a strong splenic response, possibly due to the weak hypoxemic stress experienced by the participants during their maximal apnoeic attempts (pre apnoea  $98\% \pm 1\%$  vs. post the fourth apnoeic bout  $99\% \pm 0\%$ ). Contrastingly, later work by Robertson et al. [19] documented a significant improvement in 400 m swim performance when the time-trial was preceded by a standardised warm-up and a series of three maximal apnoeas ( $275.79 \pm 12.88$  s) compared with control ( $278.66 \pm 13.31$  s,  $P=0.035$ ) but were comparable to the warm-up only protocol ( $276.01 \pm 13.52$  s), reiterating the importance of warming up. More recently, Bourdas and Geladas [2] demonstrated that a series of 5 maximal static apnoeic repetitions combined with face immersion in cold water ( $12\text{ }^{\circ}\text{C}$ ) was efficacious in improving performance ( $49.2 \pm 4.8$  s) during a time to exhaustion cycling test (i.e., intensity corresponding to 150% of peak power output) compared with control ( $44.8 \pm 8.1$  s). However, similarly to Robertson et al. [19], no differences were recorded in haemoglobin nor haematocrit concentrations following the apnoeic repetitions.

To date, no study has investigated the effect of a serial apnoeas on running performance in competitive athletes. Furthermore, to the best of our knowledge no study exists that has evaluated whether the efficacy of breath-holding on endurance performance could be affected by the total duration of the exercise intervention. In this regard, retrospective comparison of the currently available studies suggests that pre-exercise apnoeas may be more beneficial in shorter than longer duration endurance activities [2, 3, 21], with only Robertson et al. [19] demonstrating a significant positive effect of pre-exercise apnoea in comparatively longer trials.

The contradictory nature of previous studies and the lack of studies related to running identify the need for further research into the application of pre-performance apnoeas. As such the aim of this study was to identify whether a series of maximal apnoeas prior to a running time trial is effective in improving performance. Furthermore, this study attempts to determine whether the distance of the time trial impacts upon the results.

## Methods

### Experimental Design

A randomized, repeated measure cross-over design was used for this study. All testing took place on the Leeds Beckett University 400 m running track. Participants attended the track on two separate occasions performing two conditions. During one visit participants performed a warm-up only (WO) (control condition) and on the other occasion a warm-up with apnoeas (WA) with the order of the testing based on a counterbalance of participants at each testing session to minimise the effect of environmental conditions and order effects. Testing was scheduled to take place at a similar time of day for both conditions no more than seven days apart and in similar weather conditions. Participants were requested to follow similar training and dietary patterns on the days before the testing with no food (2 h) and caffeine (that day) prior to testing [8]. The testing took place during the competitive season. A distance of 200–1000 m was selected for the trial by participants based on their preferred competitive distance of 100 m, 200–1500 m respectively. These distances were selected to allow comparison between sprint (200 m) and middle distance (1500 m) runners and to allow participants who specialised in 1500 m to run an “under distance” time trial—giving maximum effort but not causing the degree of fatigue associated with full distance trials that might affect subsequent training or competition as the testing was performed “in season”.

### Subjects

Seventeen participants volunteered for this study and were stratified into two groups based on preferred competitive distance, 200 m [9 participants (5 males, 4 females)] and 1000 m [8 participants (3 males, 5 females)]. All participants were competitive athletes of a minimum of county (regional) standard. The study was approved by the Leeds Beckett Carnegie School of Sport Ethics Committee (ethics number: 36549) and all participants completed written informed consent prior to testing. Participants were free from injury and identified as low risk using the ACSM 2017 screening guidelines.

### Procedures

On arrival the participants' stature (Harpenden Stadiometer, Holtain Ltd, Pembrokeshire, UK) and body mass (Seca 709, Seca Ltd, Birmingham, UK) were assessed. Thereafter, the participants underwent a 20 min resting period following baseline measurements of heart rate (HR) (Polar FT1, Polar Electro, OY, Finland) and peripheral oxygen saturation

(SpO<sub>2</sub>) (Nonin 8500 Hand Held Digital Oximeter, Plymouth, USA) along with fingertip blood samples for the analysis of blood lactate (BL) (YSI2300 stat plus, Yellow Springs Instruments, USA), haemoglobin (Hb) (HemoCue, Radiometer Group, Sweden) and haematocrit (Hct) (Hawksley Microcentrifuge Nottingham, United Kingdom). Finally, splenic volume was determined by measuring the spleen's length (L), width (W) and thickness (T) (MindRay DP-50, Shenzhen MindRay Bio-Medical Electronics Co., Ltd., Shenzhen, China) and calculated using the Pilström formula [ $L\pi(WT-T^2)/3$ ] [6].

Following resting measurements, the participants then performed either the WO or WA protocol. During both protocols the participants performed their own pre-race warm up and were instructed to use the same routine for both testing conditions. Following completion of their warm-up, cardiovascular parameters, haematology and spleen volume were evaluated. Thereafter, over a 20-min period the participants: (i) remained seated for the entirety of this period whilst breathing normally to simulate a pre-competition holding period (WO protocol) or (ii) completed a series of five, dry, static apnoeas in a seated position, each separated by a two minutes resting period [5, 19], whilst wearing a nose clip to avoid micro breathing protocol (WA protocol). This apnoeic modality was used to provide a more practicable technique that could potentially be adapted by athletes in a competition setting, such as during a "call room" scenario [19]. At the end of WO and immediately after each successive maximal apnoeic attempt splenic volume was assessed, a fingertip blood sample (haemoglobin, haematocrit and blood lactate) was collected; heart rate and SpO<sub>2</sub> were noted.

Following the 20-min period (WO & WA), the participants transitioned immediately from the testing area to the start line, with the time-trial commencing within five minutes from completing the WO or WA protocol. The time was recorded by two timekeepers with the average time of the two measurements being used for analysis. Following completion of their respective time-trial cardiovascular parameters, haematology and splenic volumes measurements were performed.

## Statistical Analysis

Statistical analysis was performed using SPSS Statistics (version 26: SPSS Inc, Chicago, IL), with significance set at  $P < 0.05$ . The Shapiro-Wilk test was used to assess normality, whereas homogeneity was assessed using Levene's test. Sphericity was evaluated using Mauchly's test of sphericity; for instances where the assumption of sphericity was violated, the Greenhouse-Geisser correction was applied. Repeated measures ANOVAs with Tukey's post-hoc tests

were used to assess within-group differences for baseline measurements and other timepoints for breath-hold duration, HR, SpO<sub>2</sub>, haemoglobin, haematocrit, blood lactate and splenic volume. Two-way ANOVAs were used to assess temporal differences in collection time points between conditions. Pair-sample t-tests were used to compare the time-trial performances between conditions (i.e., WO vs. WA) two conditions were being compared. All graphs were produced using GraphPad Prism (GraphPad Software, Prism 9.0, San Diego, CA 92,108). Unless otherwise stated, data are reported as mean  $\pm$  SD, with significance accepted at  $P < 0.05$ . Raw data for this study are not publicly available to preserve individuals' privacy under the European General Data Protection Regulation and in accordance with the data management plan that was outlined in the participant information sheet at the point of consent.

## Results

### Performance

During the 200 m time-trial, WA resulted in a significantly faster performance ( $27.51 \pm 3.49$  s;  $P = 0.009$ ) compared with WO ( $27.96 \pm 3.34$  s), whereas no differences were observed in the 1000 m running trials (WA,  $211.10 \pm 26.18$  s; WO,  $215.82 \pm 25.13$  s,  $P = 0.120$ ) (Figs. 1 and 2). Across both conditions the average 200 m TT was  $25.32 \pm 1.33$  s for males and  $30.75 \pm 1.53$  s for females, 1000 m TT was  $197.03 \pm 18.24$  s for males and  $222.34 \pm 21.09$  s for females (Fig. 1).

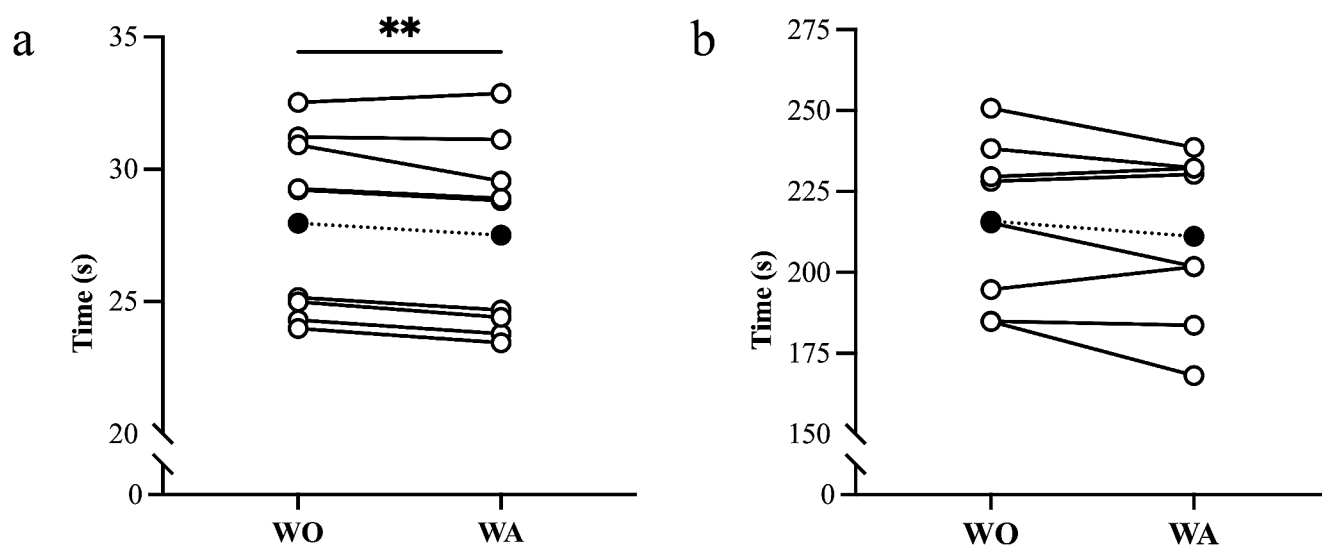
### Apnoea Duration

There was a significant increase in apnoea duration throughout the apnoea protocol with each apnoea being significantly ( $P < 0.05$ ) longer than the preceding apnoea in both groups (Table 1).

### Heart Rate

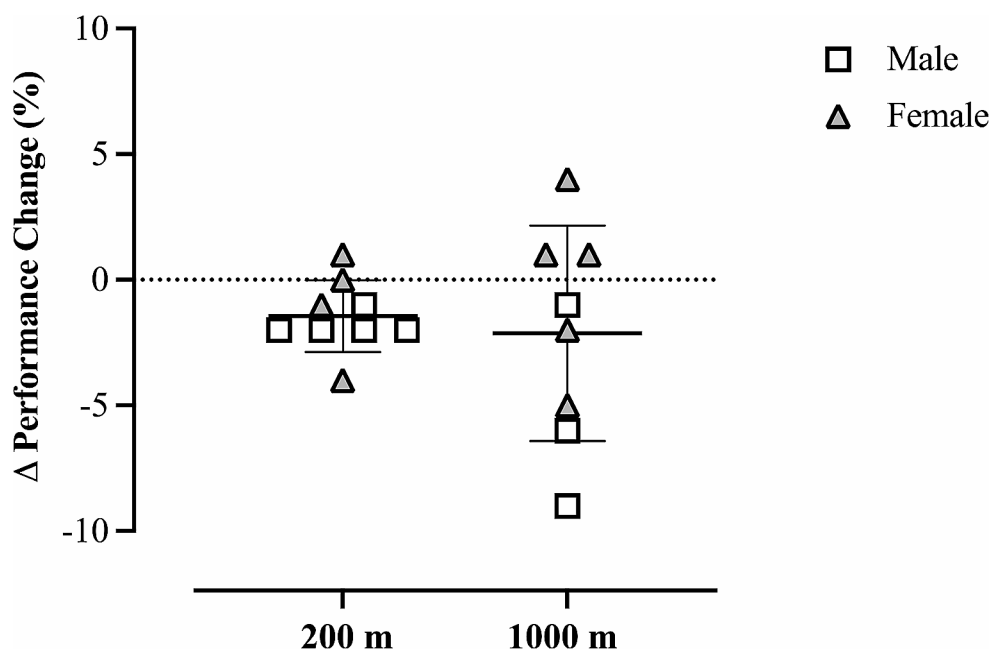
Significant differences in heart rate were recorded during both groups (200 m; 1000 m) and protocols (WO,  $P \leq 0.0001$  and WA,  $P \leq 0.0001$ ) (Table 2), whereas no differences were documented between the two interventions in either group ( $P \geq 0.069$ ).

In the 200 m group, heart rate was significantly higher than baseline compared with the warm-up (WO,  $P < 0.001$ ; WA,  $P < 0.002$ ) and post-trial (WO,  $P < 0.001$ ; WA,  $P < 0.001$ ) (Table 1). In the 1000 m group, heart rate was significantly elevated from baseline compared with warm up ( $P = 0.016$ ) and post-trial ( $P < 0.001$ ) in the WA protocol



**Fig. 1** Individual (white dots) and mean (black dots) time-trial performances following the control and apnoeic interventions during the 200 m (a) and 1000 m (b) trials. \*\* denotes between-protocol significance ( $P < 0.05$ ). *WO* warm-up only, *WA* warm-up and apnoeas

**Fig. 2** Mean ( $\pm$ SD) and individual [white rectangles (male) and grey triangles (female)] delta performance changes between *WO* and *WA* for the 200 m and 1000 m trials. *WO* warm-up only, *WA* warm-up and apnoeas



and compared with pre- and post-trial ( $P = 0.023$ ;  $P < 0.001$ , respectively) in the *WO* protocol.

During apnoeic protocol, a significant increase in heart rate was an overall affect observed in the 1000 m group ( $P = 0.013$ ) but not the 200 m ( $P = 0.138$ ), with *post hoc* analyses identifying no significant differences. No differences were recorded in the post-apnoeic heart rate levels between the two groups ( $P = 0.737$ ) (Table 2).

### Peripheral Oxygen Saturation

SpO<sub>2</sub> was lower than baseline in the 1000 m (*WO*,  $P = 0.306$ ; *WA*,  $P = 0.013$ ) but not the 200 m group (*WO*,

$P = 0.06$ ; *WA*,  $P = 0.277$ ). Specifically, during the *WA* protocol, pre-trial SpO<sub>2</sub> was significantly lower than baseline ( $P = 0.016$ ) (Table 2). Moreover, in the 1000 m but not the 200 m ( $P = 0.183$ ) group there was a significant difference between the two protocols ( $P = 0.002$ ), with a lower SpO<sub>2</sub> being documented pre-trial in *WA* ( $P = 0.043$ ) than the *WO* protocol (Table 2). There was no significant difference between groups ( $P = 0.950$ ).

During the apnoeic protocol, a significant reduction in SpO<sub>2</sub> was only documented in the 200 m group ( $P = 0.015$ ) while no differences were observed in the 1000 m group ( $P = 0.078$ ) (Table 2). Notably, SpO<sub>2</sub> was significantly lower than baseline during the fourth apnoeic repetition

**Table 1** Breath-hold durations, cardiovascular and splenic responses for each successive maximal static apnoeic attempt for both groups

Variable	Baseline		Apnoea 1		Apnoea 2		Apnoea 3		Apnoea 4		Apnoea 5	
	200 m	1000 m	200 m	1000 m	200 m	1000 m	200 m	1000 m	200 m	1000 m	200 m	1000 m
Breath-hold duration (s)	-	-	51 ± 14	56 ± 17	68 ± 19*	74 ± 19*	78 ± 22*	84 ± 18*	86 ± 25*	100 ± 23*	104 ± 26*	113 ± 31*
Heart rate (beats/min)	68 ± 12	58 ± 12	75 ± 12	72 ± 21	73 ± 12	68 ± 15	74 ± 9	71 ± 15	80 ± 15	75 ± 17	77 ± 12	71 ± 18
SpO <sub>2</sub> (%)	97 ± 1	98 ± 1	96 ± 3	93 ± 10	96 ± 3	94 ± 6	94 ± 3	94 ± 5	92 ± 3*	90 ± 9	92 ± 6	88 ± 7
Splenic volume (mL)	219 ± 78	234 ± 93	199 ± 65	196 ± 70	212 ± 77	202 ± 74	196 ± 61	192 ± 73	180 ± 59	164 ± 53*	196 ± 65	149 ± 41

Data are mean ± SD. Significant ( $P < 0.05$ ) difference from baseline is denoted as \*. SpO<sub>2</sub> peripheral oxygen saturation

( $P = 0.021$ ) (Table 2). Moreover, there were no between-group differences ( $P = 0.433$ ).

### Splenic Volume

Splenic contractions were evident in both groups [200 m (WO,  $P < 0.001$ ; WA,  $P < 0.001$ ); 1000 m (WO,  $P = 0.012$ ; WA,  $P = 0.035$ )]. Notably, a significant reduction was observed from baseline (200 m, WO,  $250 \pm 83$  mL, WA,  $219 \pm 78$  mL; 1000 m, WO,  $228 \pm 77$  mL, WA,  $234 \pm 93$  mL) to post time-trials across both distances [200 m (WO,  $224 \pm 58$  mL,  $P < 0.001$ ; WA,  $196 \pm 65$  mL,  $P < 0.001$ ); 1000 m (WO,  $204 \pm 79$  mL,  $P = 0.048$ ; WA,  $149 \pm 41$  mL,  $P = 0.014$ )] and protocols, with the only exception of a significant decrease ( $149 \pm 41$  mL;  $P = 0.045$ ) also being denoted after the apnoeic intervention (Pre-Trial) in the 1000 m group. Furthermore, there were no between-protocols (200 m,  $P = 0.165$ ; 1000 m,  $P = 0.081$ ) nor group ( $P = 0.307$ ) differences.

### Haemoglobin

Haemoglobin was significantly elevated from baseline (200 m, WO,  $146 \pm 15$  g/dL, WA,  $144 \pm 20$  g/dL; 1000 m, WO,  $138 \pm 15$  g/dL, WA,  $143 \pm 8$  g/dL) during both protocols in the 1000 m (WO,  $P = 0.018$ ; WA,  $P = 0.035$ ) but only in the WA protocol ( $P = 0.003$ ) in the 200 m group. In the 1000 m group, haemoglobin was significantly higher than baseline compared with pre-trial ( $145 \pm 12$  g/dL,  $P = 0.039$ ) and post-trial concentrations ( $149 \pm 17$  g/dL,  $P = 0.028$ ) during the WO protocol, while in the WA protocol haemoglobin was only higher than baseline compared with the post-trial concentrations ( $154 \pm 11$  g/dL,  $P = 0.011$ ). Moreover, in the 200 m WA protocol, haemoglobin concentrations were significantly higher than baseline after the apnoeic intervention ( $151 \pm 11$  g/dL,  $P = 0.041$ ) and post-trial ( $159 \pm 14$  g/dL,  $P < 0.001$ ). No differences were denoted between-protocols (200 m,  $P = 0.105$ ; 1000 m,  $P = 0.236$ ) nor groups ( $P = 0.633$ ).

### Haematocrit

Haematocrit was only significantly higher than baseline ( $43\% \pm 3\%$ ,  $P < 0.001$ ) during the WO protocol in the 200 m group. Notably, haematocrit post-trial concentrations ( $47\% \pm 4\%$ ,  $P = 0.001$ ) were significantly higher than baseline. However, there were no between-group ( $P = 0.667$ ) nor protocol (200 m,  $P = 0.145$ ; 1000 m,  $P = 0.845$ ) differences in haematocrit concentrations.

**Table 2** Cardiovascular and blood lactate responses during both protocols and interventions

<b>Warm Up Only</b>								
Variables	Baseline		Warm Up		Pre-Trial		Post-Trial	
	200 m	1000 m	200 m	1000 m	200 m	1000 m	200 m	1000 m
Heart Rate (beats/min)	71±8	62±13	105±15*	71±20	85±12	73±11*	140±17*	125±23*
SpO <sub>2</sub> (%)	97±1	97±1	95±2	95±4	96±2	97±2	95±3	94±4
Blood Lactate (mmol/L)	1.3±0.8	1.8±1.3	3.0±2.5	2.3±0.8	1.0±0.4	2.1±1.7	9.7±4.4*	9.7±4.2*
<b>Warm Up and Apnoea</b>								
Variables	Baseline		Warm Up		Pre-Trial		Post-Trial	
	200 m	1000 m	200 m	1000 m	200 m	1000 m	200 m	1000 m
Heart Rate (beats/min)	68±12	58±12	106±13*	87±21*	77±12	71±18	127±21*	127±23*
SpO <sub>2</sub> (%)	97±1	98±1	97±2	95±6	92±6	88±7*#	94±8	97±2
Blood Lactate (mmol/L)	1.0±0.2	1.1±0.7	2.8±1.1*	3.4±1.4	1.5±1.9	1.9±1.3	10.3±3.7*	9.2±3.9*

Data are mean±SD. Significant ( $P<0.05$ ) difference from baseline is denoted as \*; between intervention difference is signified as #. SpO<sub>2</sub>, peripheral oxygen saturation

## Lactate

Blood lactate was significantly elevated from baseline during both groups and protocols ( $P<0.001$ ). In the 200 m group, blood lactate concentrations were significantly higher than baseline following the run-trials in both protocols (WO,  $P=0.001$ ; WA,  $P<0.001$ ) as well as compared with post-warm up in the WA ( $P=0.006$ ) (Table 1). Moreover, during the 1000 m group, blood lactate concentrations were significantly higher than baseline following the run-trials in both protocols (WO,  $P=0.002$ ; WA,  $P=0.004$ ) (Table 1). There were no between-protocol (200 m,  $P=0.933$ ; 1000 m,  $P=0.683$ ) nor group ( $P=0.713$ ) differences.

## Discussion

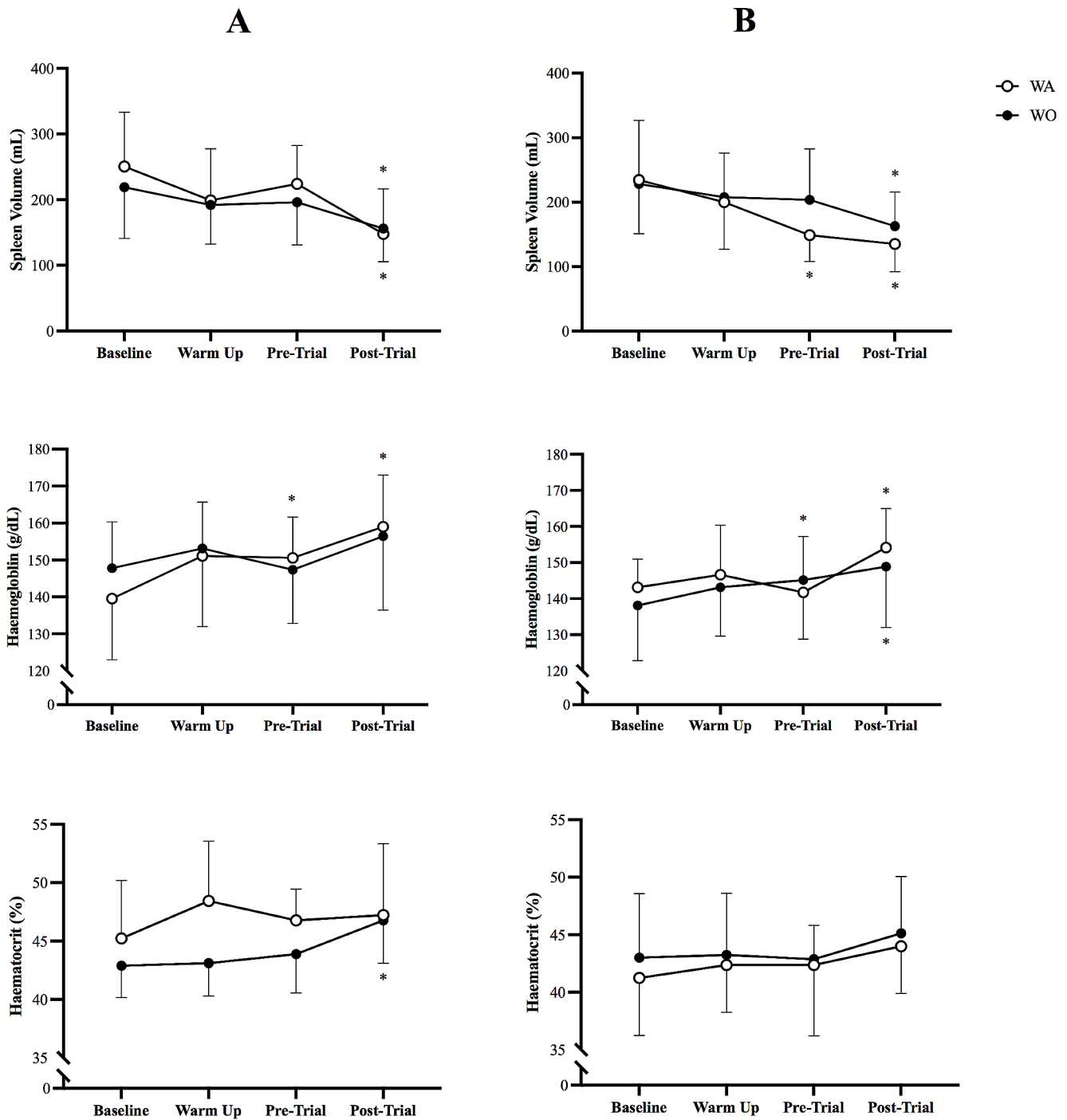
This study aimed to examine whether a series of maximal apnoeas prior to a running time trial is effective in improving performance. The key finding of this research is that five repeated maximal apnoeas significantly improved running performance in 200 m but not in 1000 m in a sample of competitive runners.

The improvement in the 200 m time-trial adds to the growing body of evidence that pre-exercise apnoeas can impact performance, as highlighted by Robertson et al. [19] and Bourdas & Geladas [2], yet the 1000 m findings supports the perspectives of Sperlich et al. [21] and Bouten et al. [4], who found no performance advantage from pre-exercise apnoea. It is interesting to note that the exercise duration of our 200 m trial ( $27.51\pm3.49$  s) and that of Bourdas and Geladas [2] ( $44.8\pm8.1$  s), were substantially shorter than our 1000 m trial ( $211.10\pm26.18$  s) and those of Robertson et al. [19] ( $275.79\pm12.88$  s), Sperlich et al. [21] ( $342\pm34$  s) and Bouten et al. [20] ( $264.8\pm14.1$  s); with only Robertson et al. [19] demonstrating a significant positive effect of pre-exercise apnoea in comparatively longer

trials. This potentially suggests that pre-exercise apnoeas may be more beneficial in shorter than longer duration endurance activities. However, further research is necessary to ascertain or refute this supposition.

Over the series of the repeated breath-holds, participants achieved progressively longer apnoeic durations, a response that is well documented across the literature [6, 11, 20]. Importantly, the gradual increases in breath-hold durations over the repeated attempts were of similar magnitude between the two groups (Table 1). This suggests that the performance gains noted in the 200 m time-trial were not attributable to differences in sustained breath-hold durations nor the hypoxaemic-dose encountered by the participants during their respective maximal attempts. In addition, although in both groups the warm-up routines elevated haemoglobin levels, these increases were only maintained following the succeeding apnoeic intervention in the 200 m group. Importantly, these increases did not concur with changes in splenic volume (Fig. 3); suggesting that factors beyond the splenic response might have also played a role in these haematological undulations. Taking into consideration that haemoglobin concentrations are prone to plasma volume changes [17, 18], it is reasonable to assume that these may have emanated from a combination of haemoglobin concentration and splenic contraction. While it is unclear what proportion of the documented increases reflect a genuine enhancement in haemoglobin content, it is tempting to speculate that these may, at least partially, have contributed to the observed improvements in performance in the 200 m group.

Serial breath-holding has been shown to transiently elevate arterial carbon dioxide levels and disrupt redox balance. These fluctuations are known to prompt vasodilation, enhance oxygen delivery to working muscles and accelerate oxygen consumption [13]; all considered advantageous from a performance perspective. Supporting this notion is that when a series of five maximal breath-holds preceded



**Fig. 3** Mean ( $\pm$  SD) splenic volume, haemoglobin and haematocrit concentrations during the WO and WA protocols for the 200 m (A) and 1000 m (B) groups. *WO*, warm up only, *WA* Warm up and apnoea. Significance ( $P < 0.05$ ) from baseline is denoted as \*

a time-to-exhaustion test as opposed to normal breathing, a greater contribution from aerobic metabolism was noted, consequently improving performance [2]. Thus, present findings might have also stem from blood gas and/or redox fluctuations, exerting a greater influence on the performance of shorter rather than longer duration endurance activities. A measure of blood gases and/or parameters related to redox

balance would have certainly provided additional insights to the mechanistic basis of our findings.

In conclusion, this study demonstrates that five repeated maximal apnoeas are capable of significantly improving a 200 m but not a 1000 m time-trial performance in competitive runners. The results suggest that the 1000 m time-trial was of sufficient duration and intensity to invoke a

significant splenic contraction and the associated haematological responses, but the inclusion of pre-exercise apnoea conveyed no physiological advantage. This study suggests that pre-exercise apnoea might be beneficial as a priming activity for short duration activities and could be employed by athletes seeking marginal gains, specifically where athletes may be confined within a 'holding area' before the performance or incorporated into warm-up activities.

**Author Contributions** All authors contributed to the study conception, design and material preparation. Data collection was performed by MB, CR, RC, MR, HC, and AE. Analyses were performed by MB and AE. The first draft of the manuscript was written by MB and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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## Declarations

**Ethical Approval** This study was performed in line with the principles of the Declaration of Helsinki. The study was approved by the Leeds Beckett Carnegie School of Sport Ethics Committee (ethics number: 36549).

**Consent to Participate** All participants completed written informed consent prior to testing.

**Consent to Publish** All participants completed written informed consent prior to testing including consent to publish.

**Competing Interests** The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

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