Title: The effect of ischemic preconditioning on maximal swimming performance

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ABSTRACT

The effect of ischemic preconditioning (IPC) on swimming performance was examined. Using a randomized, crossover design, National-and International-level swimmers (*n*=20; 14 males, 6 females) participated in three trials (Con, IPC-2h, IPC-24h). Lower-body IPC (4 x 5 min bi-lateral blood-flow restriction at 160-228 mmHg, and 5 min reperfusion) was used 2- (IPC-2h) or 24-h (IPC-24h) before a self-selected (100 m, *n*=15; 200 m, *n*=5) swimming time-trial (TT). The Con trial used a sham intervention (15 mmHg) 2h prior to exercise. All trials required a 40-min standardized pre-competition swimming warm-up (followed by 20-min rest; replicating pre-competition call room procedures) 1h before TT. Capillary blood (pH, blood gases and lactate concentrations) was taken immediately pre-and post-IPC, pre-TT and post-TT. No effects on TT for 100 m (P=0.995; IPC-2h: 64.94±8.33 s; IPC-24h: 64.67±8.50 s; Con: 64.94± 8.24 s), 200 m (P=0.405; IPC-2h: 127.70±10.66 s; IPC-24h: 129.26±12.99 s; Con: 130.19±10.27 s) or combined total time (IPC-2h: 84.27±31.52 s; IPC-24h: 79.87±29.72 s; Con: 80.55±31.35 s) were observed following IPC. Base excess (IPC-2h: -13.37±8.90 $mmol \cdot L^{-1}$; Con: -13.35±7.07 $mmol \cdot L^{-1}$; IPC-24h: -16.53±4.65 $mmol \cdot L^{-1}$), pH (0.22±0.08; all conditions), bicarbonate (IPC-2h: -11.66±3.52 $mmol \cdot L^{-1}$; Con: -11.62±5.59 $mmol \cdot L^{-1}$; IPC-24h: -8.47±9.02 $mmol \cdot L^{-1}$), total carbon dioxide (IPC-2h: -12.90±3.92 $mmol \cdot L^{-1}$; Con: -11.55±7.61 $mmol \cdot L^{-1}$; IPC-24h: 9.90±8.40 $mmol \cdot L^{-1}$), percentage oxygen saturation (IPC-2h: -0.16±1.86%; Con: +0.20±1.93%; IPC-24h: +0.47±2.10%) and blood lactate (IPC-2h: +12.87±3.62 $mmol \cdot L^{-1}$; Con: +12.41±4.02 $mmol \cdot L^{-1}$; IPC-24h: +13.27±3.81 $mmol \cdot L^{-1}$) were influenced by swimming TT (P<0.001), but not condition (all P>0.05). No effect of IPC was seen when applied 2- or 24-h before swimming TT on any indices of performance or physiological measures recorded.

Key words: Time-trial, lactate, blood gases, ergogenic aid

INTRODUCTION

During international swimming events athletes are required to perform two to three maximal efforts following months or even years of training and preparation, with marginal differences of <0.5% separating medal and non-medal positions (e.g. difference between sixth and third place in the men's and women's 100 m at World Championships; FINA, World Championship results 2017 - 8). In addition to the benefits of training, previous research has shown the importance of competition warm-up intensity (24), timing of warm-up (36) and use of active heating and land-based activation exercises (21, 22) as competition-day strategies to improve subsequent swimming performance. Ischemic preconditioning (IPC), involving cycles of ischemia and reperfusion achieved through the application of cuffs to the arms or thighs (11), has also been reported to improve indices of athletic performance when used between 15 mins and 8h before performance assessments (12).

The benefits of IPC to improve athletic performance have been previously observed in time to exhaustion (e.g. 9), anaerobic specific performance tests (e.g. 14) and repeated sprint ability (e.g. 26). It has been reported that IPC induces acute vascular adaptations, resulting in local vasodilation and enhanced blood flow (34). Consequently, enhanced functional sympatholysis may speed and increase oxygen extraction by means of matching demand with supply (13), facilitating an increased aerobic contribution during subsequent exercise. Reports suggest that IPC can cause a faster uptake of acetyl coenzyme A (acetyl-CoA) by mitochondria thus maintaining lactate accumulation at a metabolically acceptable level due to greater contribution of aerobically generated adenosine triphosphate (ATP) for exercise (14). Recruitment of higher order motor units via enhanced central motor efferent command also results from IPC (4), allowing for exercise to be completed beyond the individual's critical threshold by increasing or maintaining the rate of force development and improving subsequent performance.

However, only one study (31) relating to sports performance has differentiated between the observed early and late phase of IPC reported within the clinical literature, implementing IPC 24h prior to a 5

km running time trial (TT). Research suggests that there are two phases resulting from IPC; the early phase which begins soon after reperfusion and lasts 3-4h, whereas the late phase starts 12–24h after IPC (16) and last 48–96h (27, 33). The release of endogenous substances is thought to stimulate post-translational modifications in proteins within the early phase, whereas in the late phase this leads to synthesis of new proteins and altered gene expression (34). Accordingly, owing to the timing of pre-competition practices and regulations in athletic competitions (e.g., the use of pre-competition call-rooms within 20 min of competition starting), the late phase of IPC may offer another practical option, to coincide with competition timings to further optimize swimming performance on the day of competition.

With a specific emphasis on swimming performance, IPC may be beneficial for 100 to 400 m swimming performance due to the resultant increase in contribution of ATP generated from the aerobic system (28). To date, four studies (7, 14, 17, 20) have identified a positive effect of implementing IPC prior to swimming performance. For example, Jean-St-Michel et al. (14) reported that five min of ischemia followed by five min of reperfusion, repeated for four cycles, implemented 45 min prior to 100 m swimming TT improved personal best swimming times by 1.1%. Most recently, Lisbôa et al. (17) applied IPC 1h, 2h and 8h preceding a 50 m TT performance, with performance improvements of 1.0% and 1.2% in 2h and 8h conditions, respectively. The previous research relating to IPC and swimming performance has investigated the effects of the early phase of IPC on performance as application has been <12h prior to performance. However, for short duration events (i.e. 10-90 s), a recent meta-analysis showed that a longer duration between IPC and exercise resulted in a higher effect size; suggesting that IPC may be dependent on the timing of the preconditioning strategy relative to the start of subsequent performance (30). Research is yet to investigate if the delayed phase of IPC can enhance swimming performance when applied at least 12h prior to competition, a strategy which may be attractive for coaches and swimmers. Consequently, the purpose of this study was to investigate the impact of IPC on swimming TT performance 2h (early phase) and 24h (late phase) after eliciting IPC in competitive swimmers.

METHOD

EXPERIMENTAL APPROACH TO THE PROBLEM

Twenty National and International-level swimmers participated in a randomized, crossover design that involved three sessions (Con, IPC-2h, IPC-24h) separated by seven days. Timing of IPC completed in conditions were implemented in line with previous research complete by Seeger et al. (31) and Lisbôa et al. (17). Occlusion cuffs were applied bi-laterally at the most proximal point of each thigh and intermittently inflated to an individualized cuff pressure determined from thigh girth and resting blood pressure for a total of 40 min in IPC-2h and IPC-24h. In Con, cuffs were applied for the same duration (total 40 min), however cuff pressure was inflated to 15 mmHg. A self-selected (100 or 200 m) swimming TT (assessing total time, 50 m split times, stroke count; SC, and stroke rate; SR, time underwater off starts and turns) followed intervention administration and physiological markers (pH, blood gases and lactate concentrations) were assessed at pre-IPC, post-IPC, pre-TT and post-TT.

SUBJECTS

Following ethical approval from Swansea University ethics committee, twenty (6 females, 14 males) National- and International-level swimmers (age; 20±2 y, mass; 71.1±9.6 kg, stature; 178.4±9.6 cm, Training experience; 9.6±2.7 y) participated in the study. All subjects had qualified for, and competed at British swimming National competitions. Subjects were informed of the experimental procedures, the purpose and possible risks associated with the study, and provided written informed consent before participation.

PROCEDURES

After familiarization, participants were required to attend the testing venue on three occasions (Con, IPC-2h, IPC-24h) in a randomized order. Main trials were performed in an enclosed 50 m swimming pool within the subject's normal training environment. To minimize the effects of biological rhythms, the timing of measurements was consistent between trials. To control for varying levels of weekly fatigue, testing was conducted on the same day of the week in a stable, maintenance phase of training.

Subjects were required to refrain from alcohol and intense physical exercise in the 24h preceding trials and between IPC and swimming TT performance.

On arrival for main trials, subjects were required to rest for 10 min to allow for resting blood pressure to be recorded (Omron Healthcare, Europe; systolic >140 mmHg and/or diastolic >90 mmHg precluded further study involvement). Once blood pressure was recorded, thigh girth was measured for determination of cuff pressure and a capillary blood sample was taken. Occlusion cuffs were then applied to the most proximal point of the thighs, with subjects assuming a supine position. The cuff (10 cm) contained a pneumatic bag along its inner surface that was connected to a pressure gauge and manually inflated to either 15 mmHg (Con) or an individualized cuff pressure (IPC-2h, IPC-24h) for a total of 40 min consisting of four cycles of five min occlusion and five min reperfusion. The individualized cuff pressures were calculated from Loenneke et al. (18) with values ranging from 160 to 228 mmHg. Cuff pressure was 15 mmHg in the Con condition; based on previous research showing that 10-20 mmHg (e.g. 1, 14, 26) caused no alteration to the arterial inflow but allowed increased control over the placebo effect as cuffs were worn in both conditions.

Following the completion of the IPC protocol, subjects rested accordingly for 24h or 2h; intense physical activity was restricted during the 24h and all subjects arrived at the swimming pool and rested for 3h prior to TT regardless of the condition, cuffs were applied during this period for IPC-2h and Con. A standardized race swimming warm up (40-min) was performed 1h prior to a swimming TT and a 20-min post-warm-up rest period at the swimming pool replicated pre-competition call room requirements. This was immediately followed by a maximal swimming TT (100 m: n=15, 200 m: n=5), completed on the subjects' chosen stroke, in accordance with FINA rules. Subjects completed the TT individually, starting from a block and taking off after an audible starting signal. Rating of perceived exertion was recorded using the Borg (2) scale on completion of the race. From the TT, SR, SC, 50 m split times, time underwater off the start and turns and total time were calculated retrospectively from video recordings. Equation 1 was used to determine SR; for each 25 m of the TT SR was calculated, the mean ± SD was then calculated for each 50 m. To ensure acceptable reliability

of the SR measurement, intra-observer tests were completed. The analyst viewed two randomly selected TT performances ten times over a two-week period under the same conditions. The coefficient of variation (CV) was calculated to identify the measurement error; this resulted in a low, acceptable percentage of error (CV = 0.2%).

Equation 1: Stroke rate = $\frac{\text{(Number of complete strokes over 25 m x 60)}}{\text{(Time of hand entry 1 – time of hand entry 2)}}$

Where hand entry 1 is the first-hand entry at the start of 25 m and hand entry 2 is the hand entry at the end of 25 m, recorded in seconds.

A capillary blood sample was taken pre-IPC, post-IPC, pre-TT and post-TT to measure blood lactate, pH, percentage of oxygen saturation (sO_2%), partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), total carbon dioxide (TCO_2), bicarbonate (HCO_3) and Base Excess. This was analyzed using a portable analyser (ISTAT 1; 300G) and associated cartridges (CG4+; Abbott, point of care testing, Arbroath, UK). Prior to data collection the analyzer was calibrated according to the manufacturer's specifications and cartridges were stored as per manufacturer's instructions (2-8°C) and removed to room temperature ~5 min prior to use. The capillary blood sample was immediately expelled from the capillary tube into the sample well of the cartridge. Blood gases and pH were analyzed using these methods which have previously been compared (35) against two auto-calibrated analyzers ($r > 0.993$). Dascombe et al. (5) also confirmed intra-test reliability of the analyzer; intra-class correlation coefficients (ICC) for all analytes were observed to be strong following maximal intensity exercise (ICC = 0.77-0.95; where 0.7-0.9 deemed a strong correlation) and technical error of measurement (TEM) <15% was deemed acceptable (pH; 0.24%, blood lactate; 3.12%, all other measured blood gas parameters 2.02-8.85%).

STATISTICAL ANALYSES

All data is presented as mean ± standard deviation (SD). Following confirmation of parametric assumptions, repeated measures multivariate analysis of variance (MANOVA) with Bonferroni adjustment assessed between-trial differences for variables with multiple time points per trial (i.e. blood lactate, pH, sO_2%, PO_2, PCO_2, HCO_3 and Base Excess). One-way ANOVA assessed between-trial differences for all performance variables from the swimming TT and RPE recorded post-TT. Statistical analyses were carried out using SPSS version 22.0 (SPSS Chicago, IL) with significance being accepted at $P \leq 0.05$.

RESULTS

Exercise significantly affected blood parameters; following swimming TT, pH decreased by 0.22±0.08 in all conditions ($P<0.001$; $\eta^2 = 0.866$) (Figure 1). Blood lactate increased pre-to post-TT ($P<0.001$; $\eta^2 = 0.923$) by 12.87±3.62 mmol·L^{-1}, 12.41±4.02 mmol·L^{-1} and 13.27±3.81 mmol·L^{-1} in IPC-2h, Con and IPC-24h, respectively (Figure 1). Base excess (IPC-2h: -13.37±8.90 mmol·L^{-1}; Con: -13.35±7.07 mmol·L^{-1}; IPC-24h: -16.53±4.65 mmol·L^{-1}; $P<0.001$; $\eta^2 = 0.857$), HCO_3 (IPC-2h: -11.66±3.52 mmol·L^{-1}; Con: -11.62±5.59 mmol·L^{-1}; IPC-24h: -8.47±9.02 mmol·L^{-1}; $P<0.001$; $\eta^2 = 0.849$), TCO_2 (IPC-2h: -12.90±3.92 mmol·L^{-1}; Con: -11.55±7.61 mmol·L^{-1}; IPC-24h: 9.90±8.40 mmol·L^{-1}; $P<0.001$; $\eta^2 = 0.939$) and sO_2% (IPC-2h: -0.16±1.86 %; Con: +0.20±1.93 %; IPC-24h: +0.47±2.10 %; $P<0.001$; $\eta^2 = 0.130$) were significantly different pre-TT to post-TT. However, there were no differences between trials in any of the blood parameters ($P>0.05$).

***** INSERT FIGURE 1 NEAR HERE *****

Trial did not affect performance for 100 m ($P=0.995$; IPC-2h: 64.94±8.33 s; IPC-24h: 64.67±8.50 s; Con: 64.94± 8.24 s), 200 m ($P=0.405$; IPC-2h: 127.70±10.66 s; IPC-24h: 129.26±12.99 s; Con: 130.19±10.27) or combined total time (IPC-2h: 84.27±31.52 s; IPC-24h: 79.87±29.72 s; Con: 80.55±31.35 s). No significant effects between conditions for any of the performance variables were

observed; being, total time (P=0.723), split time for the first 50 m (P=0.968), split time for the second 50 m (P=0.874), start time (P=0.817), turn time at 50 m (P=0.924), SC for first 50 m (P=0.559), SC for second 50 m (P=0.570), SR for first 50 m (P=0.726), SR for second 50 m (P=0.988) and RPE (P=0.723) (Table 1).

***** INSERT TABLE 1 NEAR HERE *****

DISCUSSION

In this study IPC did not affect 100 or 200 m swimming performance in National-level swimmers when applied 2h or 24h prior to performance assessment. These findings, particularly for IPC-2h, oppose previous research that found IPC applied acutely improved subsequent swimming performance (7, 14, 17, 20). Consistent with previous research (31), no change in swimming performance was identified when IPC was applied 24h before the TT. Likewise, no differences were identified in physiological markers following IPC-2h or IPC-24h. Therefore, IPC applied 2h or 24h had no influence, either positive or negative, on swimming performance or physiological markers.

For short duration events (i.e. 10-90 s), a recent meta-analysis showed that a longer duration between IPC and exercise resulted in a higher effect size; suggesting that IPC may be dependent on the timing of the preconditioning strategy relative to the start of subsequent performance (30). Previous research in swimming has implemented IPC between 10 min and 8h (7, 14, 17, 20) before performance assessment and found beneficial effects; findings which contradict those reported here when IPC was applied 2h before exercise. Several methodological differences between the present study and previous literature may explain this lack of agreement in findings. Specifically, there is little consensus regarding optimal cuff pressures used in IPC as a range of pressures have been reported (i.e., 200-230 mmHg or 15-50>SBP) which are universally applied across all individuals within studies. A standardized cuff pressure may not cause the same percentage of blood flow restriction in every individual, especially considering the volume and type of tissue surrounding the blood vessels which may influence the pressure exerted on the vasculature (19). Therefore, the percentage of blood

flow restriction may affect the success of IPC as a pre-competition strategy (10). Recent research by Loenneke et al. (18) recommended the use of individualized cuff pressure calculated from thigh girth and resting blood pressure, which was adopted in the current study. However, individual blood flow restriction was not confirmed using a Doppler due to practicality, which offers a limitation to the current study as blood flow restriction was calculated in alignment with results from previous research (18), rather than according to a measured pressure. A protocol to individualise cuff pressure needs to be determined, identifying the differences between a standard cuff pressures and the use of thigh girth and blood pressure to calculate individual pressures in comparison to Doppler assessment. The results of these three methods to determine cuff pressure need to be identified and the resultant effect on performance tested to establish recommendations for practical use.

To explain the current results, another methodological difference should be considered regarding the location of the cuff, with application previously reported on the lower or upper body. The present study applied occlusion cuffs to the thighs which contrasts previous research in swimming whereby cuffs were applied to the upper body (7, 14, 17, 20). Although limited research still exists on the working mechanism of IPC and athletic performance, it has been suggested that IPC induces a systemic change in blood flow through a change in sympathetic activity. Due to the nature of swimming and controlled breathing, which can result in exercise induced arterial hypoxemia, decreased pH (3, 32) and consequently a significant contributor of fatigue (25), a systemic increase in blood flow and oxygen delivery could be speculated to improve performance, reducing hypoxemia and metabolic acidosis. However, in the current study no differences were identified between conditions in the physiological measures. Alternative research has suggested that IPC may also cause local changes in the muscle at the site of the cuff (e.g. increase oxygen uptake or change in mitochondrial activity) which may contribute to an increase muscle oxygenation (13, 15, 29). In swimming, the contribution of propulsive force is approximately 90% for the upper extremities (6, 23), therefore, the local changes achieved by application of the cuffs to the upper limbs, may increase effectiveness of limb IPC to improve swimming performance. In comparison to previous results applying cuffs to the thighs to induce a systemic response, this may help to explain the inconsistency

in the current results, highlighting this as an area warranting further investigation to determine the impact of systemic versus local blood flow restriction on athletic performance.

To date, one study has examined the use of IPC applied 24h prior to performance to determine if the late phase of IPC, originally used in a clinical setting, may also improve athletic performance. The current study replicated research completed by Seeger et al. (31) but within swimming, with the only other methodological difference being individualizing of cuff pressures. Similarly, no difference in performance time between conditions was identified. However, results from the current study were not consistent with previous research investigating IPC in swimming as previously a benefit has been identified in the early phase (10 min – 2h) within the literature which was not consistent in our study. Therefore, methodological differences could have influenced these findings as stated above regarding cuff location, consequently IPC applied 24h prior to performance should be further investigated in swimming while ensuring that cuffs are applied to the upper body.

In conclusion, the current study demonstrated swimming TT performance of 100 or 200 m was not influenced when it was preceded 2h or 24h by four cycles of IPC, at an individualized cuff pressure. Speculatively, this may have been due to the difference in cuff placement on the lower limbs as opposed to upper limbs as in previous IPC and swimming research. Therefore, the use of IPC 24h prior to swimming TT performance should be investigated with cuffs applied to the upper limbs to identify if the late phase of IPC can also improve performance, as this would have greater practical application completing the IPC protocol 24h before competition rather than in close proximity to the start of an athletic event.

PRACTICAL APPLICATIONS

Despite this study concluding swimming performance was not influenced by IPC applied at 2h or 24h, there are several practical points of relevance for application in sport. These results provide baseline data for the use of IPC in swimming when cuffs are applied to the thighs, identifying that this strategy had no detrimental effect on physiological responses. Most prominently, the combination of previous

research and the current study suggest recommendations for application of the cuffs to the upper body to improve swimming performance.

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474 **FIGURES AND TABLES**

475

476 **Figure 1**: Change in blood markers from pre-ischemic preconditioning (IPC) to post-IPC and Pre-
477 time trial (TT) to post-TT

478

479 **Table 1**: Performance variables from the swimming time trial (100 and 200 m combined) for the three
480 conditions

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Condition	SC 50	SC 100	SR 50 (SPM)	SR 100 (SPM)	Start (s)	Turn 50 (s)
Con	19.3±2.4	22.2±3.2	45.3±8.0	42.5±7.1	4.9±1.4	4.2±1.6
Confidence Interval	18.1-20.5	20.6-23.8	41.3-49.3	39.0-46.0	4.2-5.5	3.4-5.0
IPC-2h	18.8±2.6	21.3 ±3.2	43.9±8.1	42.3±7.4	4.9±1.4	4.2±1.7
Confidence Interval	17.5-20.0	19.7-22.8	40.1-47.7	38.9-45.8	4.3-5.6	3.4-5.0
IPC-24h	18.4±2.6	21.1±3.7	43.4±8.7	42.1±6.3	5.1±1.1	4.4±1.7
Confidence Interval	17.1-19.7	19.2-23.0	38.9-47.8	38.8-45.5	4.6-5.7	3.5-5.3

482 *SC50 = stroke count for the first 50 m, SC 100 = stroke count for the second 50 m, SR 50 = stroke
483 rate for first 50 m, SR 100 = stroke rate for second 50 m, start = time from dive start to first stroke,
484 Turn 50 = turn time at 50 m. Confidence intervals reported at ninety-five-percent.

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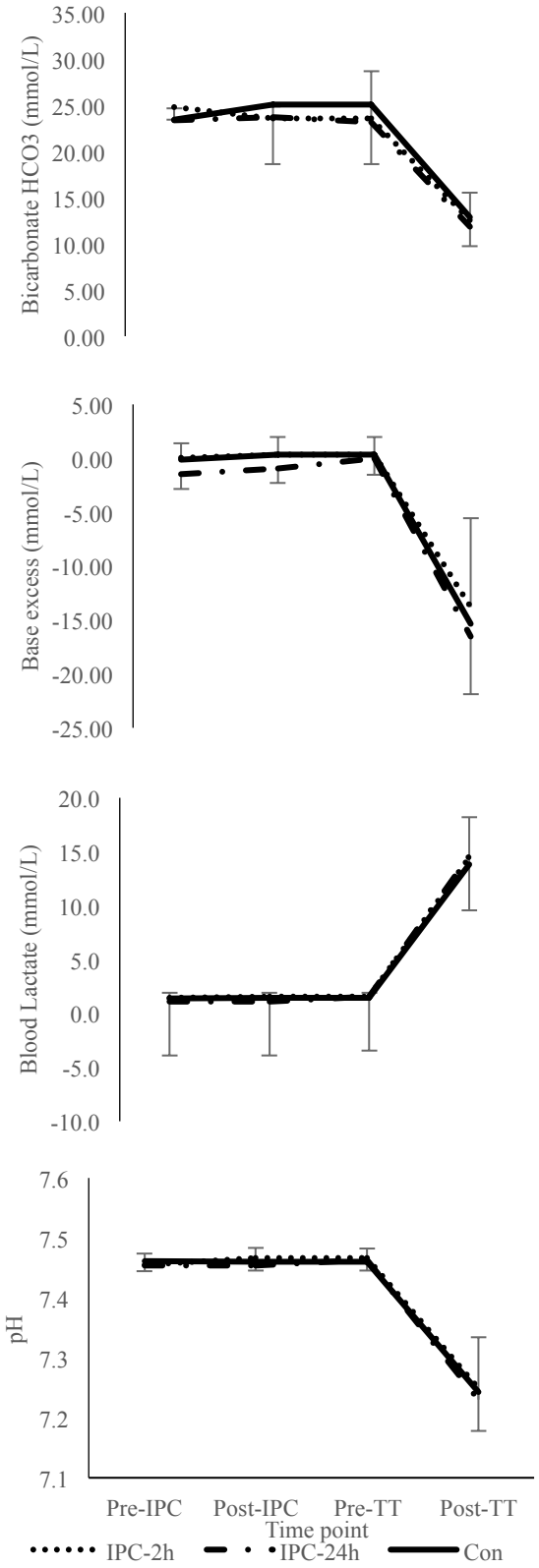
Condition	SC 50	SC 100	SR 50 (SPM)	SR 100 (SPM)	Start (s)	Turn 50 (s)
Con	19.3±2.4	22.2±3.2	45.3±8.0	42.5±7.1	4.9±1.4	4.2±1.6
Confidence Interval	18.1-20.5	20.6-23.8	41.3-49.3	39.0-46.0	4.2-5.5	3.4-5.0
IPC-2h	18.8±2.6	21.3 ±3.2	43.9±8.1	42.3±7.4	4.9±1.4	4.2±1.7
Confidence Interval	17.5-20.0	19.7-22.8	40.1-47.7	38.9-45.8	4.3-5.6	3.4-5.0
IPC-24h	18.4±2.6	21.1±3.7	43.4±8.7	42.1±6.3	5.1±1.1	4.4±1.7

Confidence Interval	17.1-19.7	19.2-23.0	38.9-47.8	38.8-45.5	4.6-5.7	3.5-5.3

*SC50 = stroke count for the first 50 m, SC 100 = stroke count for the second 50 m, SR 50 = stroke rate for first 50 m, SR 100 = stroke rate for second 50 m, start = time from dive start to first stroke, Turn 50 = turn time at 50 m. Confidence intervals reported at ninety-five-percent.



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