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The Effect of Breathing Patterns Common to Competitive Swimming on Gas Exchange and Muscle Deoxygenation During Heavy-Intensity Fartlek Exercise

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Kinesiology

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Abstract

The purpose of this study was to compare the respiratory and muscle deoxygenation (HHb) responses of regulated breathing versus free-breathing, during continuous exercise (CONLD) and intermittent 5s breath holds (BH) (CONLD-BH), intermittent 5s sprint (FLK) and combined 5s BH and sprint (FLK-BH) followed by 25s of free-breathing. Oxygen uptake ($\dot{V}O_2$) was unchanged between CONLD (2.12 ± 0.35 L/min) and CONLD-BH (2.15 ± 0.42 L/min; $p=0.116$), and FLK (2.24 ± 0.40 L/min) and FLK-BH (2.20 ± 0.45 L/min; $p=0.861$). $\Delta[Hb_{tot}]$: CONLD ($3.3 \pm 1.6 \mu\text{M}$) > CONLD-BH ($-2.5 \pm 1.2 \mu\text{M}$; $\Delta 177\%$; $p < 0.001$), but unchanged between FLK ($2.0 \pm 1.6 \mu\text{M}$) and FLK-BH ($0.82 \pm 1.4 \mu\text{M}$; $p=0.979$). $\Delta[HHb]$: CONLD ($7.3 \pm 1.8 \mu\text{M}$) > CONLD-BH ($7.0 \pm 2.0 \mu\text{M}$; $\Delta 4\%$; $p=0.011$), and FLK ($6.7 \pm 1.8 \mu\text{M}$) < FLK-BH ($8.7 \pm 2.4 \mu\text{M}$; $p < 0.001$). It is suggested that the unchanged $\dot{V}O_2$ between CONLD and CONLD-BH was supported by increased deoxygenation, reflected by decreased $\Delta[Hb_{tot}]$ and blunted $\Delta[HHb]$, via apneic-driven redistribution of blood flow away from working muscles, which is reflected by the decreased $S_{at}O_2$. However, the preserved $\dot{V}O_2$ during FLK-BH versus FLK has been underpinned by the increase [HHb].

Keywords

Apnea, Regulated Breathing, Front Crawl, Flip Turn, Deep Diving Response

Summary for Lay Audience

Breath holds (BH) during exercise restrict oxygen transport to tissues, which hinders the working muscles' ability to maintain the required energy demand. This is because oxygen is required for one of the major energy regeneration processes, aerobic metabolism. In aquatic sports such as swimming, irregular breathing and BH patterns limit gas exchange at the lungs, including oxygen uptake and carbon dioxide (CO₂) removal. Since the rate of oxygen consumption ($\dot{V}O_2$) ultimately reflects the rate of aerobic metabolism, it was expected that $\dot{V}O_2$ would be lower compared to similar exercises on land.

Near infrared spectroscopy (NIRS) can be used to measure regional oxyhemoglobin concentrations non-invasively. Deoxyhemoglobin (HHb) represents successful offloading of oxygen from hemoglobin (Hb) and myoglobin (Mb). Total hemoglobin (Hb_{tot}) reflects the number of Hb under the NIRS probe.

In the present study, we were interested in the effects of a free-breathing protocol constant load (CONLD) compared to a regulated breathing strategy common to front crawl swimming. To mimic swimming 50 m laps, repeated cycles of 5 s BHs performed every 30 s were employed. During the 25 s, breathing frequency was matched to the same period of the free-breathing protocol (CONLD-BH). In two other conditions, a periodic sprint was performed every 30 s under normal (FLK) and BH conditions (FLK-BH) during the 5 s intervals. Breathing was also regulated between FLK and FLK-BH during the non-apneic 25 s intervals. Mean $\dot{V}O_2$ was unchanged between CONLD and CONLD-BH. However, [Hb_{tot}] was lower in CONLD-BH, perhaps reflecting reduced regional blood flow to the muscle. [HHb] was also lower in CONLD-BH, however, [Hb_{tot}] was minimally affected. This suggests that the muscle under investigation (quadriceps) was relying on increased deoxygenation. The observed redistribution of blood flow away from working muscles allows deep diving athletes to reach further depths. Elite deep divers can maintain the $\dot{V}O_2$ required by their exhaustive efforts while simultaneously rerouting drastic volumes of blood to central organs.

$\dot{V}O_2$ was also unchanged between FLK and FLK-BH. This BH resolution was expressed by an increase in [HHb] reflecting greater muscle oxygenation from Hb. Therefore, both protocols resolved unchanged $\dot{V}O_2$ through increased oxygen offloading from Hb.

Co-Authorship Statement

This study was designed by Kevin Grossman, David Lim, Glen Belfry, and the data were collected by David Lim. Kevin Grossman analyzed the data and wrote the manuscript. Kevin Grossman, Glen Belfry and Juan Murias edited the manuscript.

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List of Abbreviations

A-a ΔO_2 – Alveolar-arterial difference in oxygen

ADP – Adenosine diphosphate

ATP – Adenosine triphosphate

BH – Breath hold

CO₂ – Carbon dioxide

CONLD – Continuous-load exercise

CONLD-BH – Continuous- load exercise with breath holds

CP – Critical power

CT – Critical threshold

ETC – Electron transport chain

FADH₂ – Flavin adenine dinucleotide

f_B – Frequency of breathing

F_IO₂ – Fraction of oxygen present in inspired air

FLK – Fartlek exercise

FLK-BH – Fartlek exercise with breath holds

GET – Gas exchange threshold

H⁺ – Hydrogen ion

H₂O – Water

Hb – Hemoglobin

[Hb_{tot}] – Total hemoglobin concentration

[HHb] – Deoxyhemoglobin concentration

[HbO] – Oxyhemoglobin concentration

[HHb]/ $\dot{V}O_2$ – Adjustment of [HHb] to $\dot{V}O_2$

Hz – Hertz

INT – Intermittent exercise

La⁻ – Lactate ion

LT – Lactate threshold

m – Meter

Mb – Myoglobin

min – Minute

MLSS – Maximal lactate steady state

N₂ – Nitrogen

NADH – nicotinamide adenine dinucleotide

NIRS – Near infrared spectroscopy

nm – Nanometer

O₂ – Oxygen

PCr – Phosphocreatine

P_{CO2} – Partial pressure of carbon dioxide

P_{ETCO2} – End-tidal partial pressure of carbon dioxide

P_{ETO2} – End-tidal partial pressure of oxygen

pH – Potential of hydrogen

P_i – Inorganic phosphate

PO – Power output

P_{O₂} – Partial pressure of oxygen

PPO – Peak power output

RCP – Respiratory compensation point

RI – Ramp incremental

RPM – Revolutions per minute

S – Second

S_{atO₂} – Tissue hemoglobin saturation percentage

SD – Standard deviation

SNR – Signal-to-noise ratio

TCA – Tricarboxylic acid

\dot{V}_{CO_2} – Rate of carbon dioxide elimination

V_D – Dead space volume

\dot{V}_E – Minute ventilation

\dot{V}_{O_2} – Rate of oxygen consumption

$\dot{V}_{O_{2max}}$ – Individual maximum rate of oxygen consumption

V_T – Tidal volume

VT₁ – First ventilatory threshold

VT_2 – Second ventilatory threshold

VTI – Inspiratory tidal volume

W – Watts

Chapter 1

1 « Review of the Literature »

Many popular sports require athletes to perform short, high-intensity efforts interspersed with periods of recovery. Competitive swimmers must manage their energy expenditure by pacing their work intensity across each lap, and over the course of the race. One common swimming race strategy is to kick off the pool wall after each turn, while submerged, which increases their speed, and reduces the duration of the arm work before the next turn by approximately five seconds (1). Swimming introduces another complication. Specifically, swimmers are required to perform the kicking after a turn under breath hold (BH) or apneic conditions. Moreover, the swimming stroke performed between these apneic periods may affect breathing differently. For example, back stroke affords swimmers the opportunity to breathe freely compared to front crawl, where the face is fully submerged, and breathing must be regulated with the rhythm of the stroke until the length of the pool is completed and the next turn is initiated (~25 s).

Swimming competition and training may also require the execution of these BHs at various kicking intensities. Previous work has observed the impact of the aforementioned, repeated cycles of five second BHs and 25 s of free breathing on gas exchange and muscle deoxygenation under free breathing conditions (2). The present study aimed to identify the respiratory and muscle deoxygenation responses to a similar exercise and BH paradigm, but under regulated breathing constraints during the 25 s between these intermittent five second apnea periods. This chapter compiles a review of the relevant literature associated with this BH paradigm and discusses the energy systems, exercise intensity domains, apnea and hypoxia, intermittent exercise, and the integrated response of apnea and exercise relevant to these power outputs (PO) and ventilatory patterns of swimming. It concludes with an overview of the background theory and equipment related to pulmonary gas exchange and muscle oxygenation data collection.

1.1 « Energy Systems »

During exercise, the metabolic demands of the exercising muscles and other active body systems (e.g., the brain) must be met by three energy systems: PCr-ATP, anaerobic glycolysis, and oxidative phosphorylation. While their activity depends on several factors, including fuel supply, hormones, exercise intensity and exercise duration, these energy systems work concertedly to sustain the necessary energy demand. These energy systems will be enumerated and explained within the context of a continuous intensity (constant-load, CONLD) bout of exercise.

1.1.1 Phosphocreatine (PCr)-ATP

The PCr-ATP system involves substrate-level phosphorylation of ADP into ATP (Figure 1). The reaction is reversible and is catalyzed by creatine kinase (3). The system is considered anaerobic because it occurs independent of O₂ (3). At the onset of exercise, [PCr] in the muscle depletes rapidly to regenerate ATP in response to the disrupted internal equilibrium in muscle – higher [ADP] and lower [ATP] from repeated contractions (3). In fact, by the first 10 s of high intensity exercise, most of the skeletal [PCr] is depleted (3), which may severely hinder high power generation (4). Rest periods allow for resynthesis of PCr (5, 6), that facilitates subsequent peak power output (PPO) and prolongs the onset of fatigue and impaired power generation (7). In the present study, the ATP-PCr system was a primary contributor to the maintenance of ATP generation during the first 10-15 s of cycling exercise.

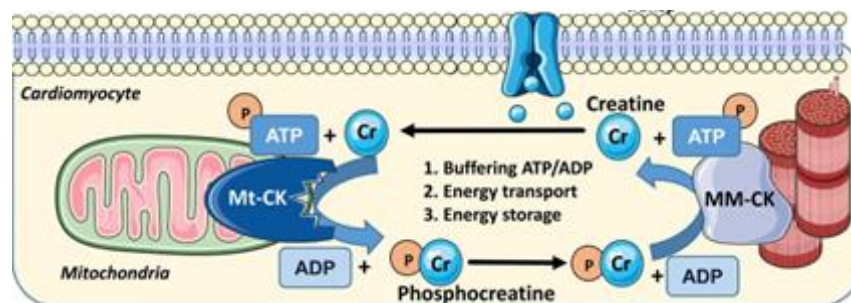


Figure 1. Illustration of the biogenesis of creatine, and ATP regeneration of the ATP-PCr system (8).

1.1.2 Anaerobic glycolysis

Anaerobic glycolysis is the next fastest generator of ATP that uses substrate-level phosphorylation (Figure 2). Conventionally, it has been described as a complex series of reactions that catalyze the breakdown of glucose or glycogen, and their derivatives (e.g., glucose 6-phosphate), into pyruvate. However, under both aerobic and anaerobic conditions, some pyruvate is further catalyzed into lactate (La^-), demonstrating that La^- is an end-product of glycolysis (9). At the onset of high-intensity exercise, anaerobic glycolysis accounts for most ATP production, after PCr depletion (10). This anaerobic contribution is negligible during moderate-intensity work (11). Moreover, glycolytic rate decreases over the exercise duration at all intensities (11). The rapid activation of glycolysis early in exercise accounts for the “rapid component” of the profile of $\dot{V}\text{O}_2$ (11).

As a product of anaerobic glycolytic phosphorylation during exercise, La^- can be fully oxidized and may even be the preferred metabolic substrate over glucose (12). It has been described as the “link” between anaerobic and aerobic metabolisms (9), as La^- may be shuttled out of muscle cells and into the blood, where it acts as a fuel source elsewhere (9). For example, the heart (13), kidneys (14) and brain (15) have been shown to oxidize La^- . La^- is also a cell signaling molecule (9) that upregulates genes related to

mitochondrial biogenesis (16, 17) and inhibits lipolysis in adipocytes (18). However, if the rate of La^- production is greater than shuttling and removal, as in heavy-intensity exercise, then La^- and H^+ accumulate in the muscle and diffuse into the blood. The combination of La^- and H^+ production intrinsic to glycolysis contributes to fatigue (9). The accumulation of H^+ , which is related to low pH (metabolic acidosis), and inorganic phosphate (P_i) inhibits the cross-bridge cycling of actin and myosin (19, 20). It was previously demonstrated in-vitro that the addition of La^- ions to an electrically charged muscle cells attenuated fatigue (21), possibly through mitigation of the inhibition of cross-bridge formation (9). Further, metabolic acidosis induces a slowed attachment and detachment rate of myosin to actin, which would decrease the potential for force generation (22). Moreover, high $[\text{P}_i]$ in an acidic environment may stimulate myosin head detachment, which would also contribute to fatigue (22). However, some have suggested that metabolic acidosis associated with La^- accumulation may not significantly contribute to fatigue, but rather elicit positive adaptations (12). For example, La^- may improve muscle blood flow and stimulate O_2 offloading from Hb (23). However, the greater Hb denaturation (24) and muscle proteolysis (25) while under acidic stress would seem to override these positive effects of La^- accumulation. It has also been suggested that H^+ ions are produced parallel to, instead of in consequence of, glycolysis (9, 12). In the current study, La^- was measured before and after exercise to estimate the anaerobic glycolytic contribution to the different exercise conditions.

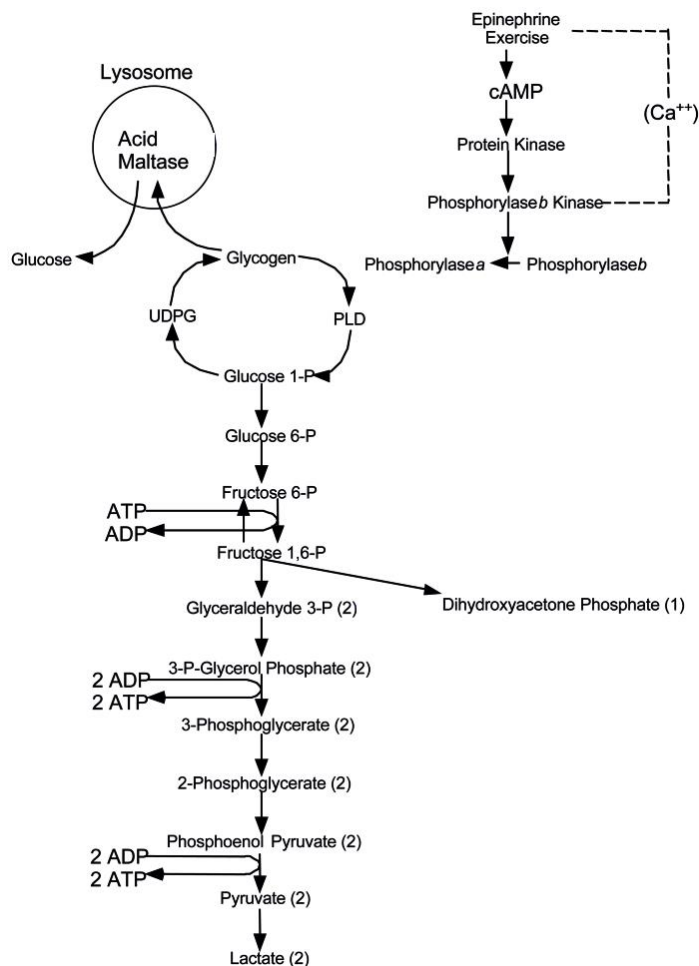


Figure 2. A mechanistic diagram of anaerobic glycolysis in muscle. Intramuscular glycogen and cytosolic glucose are the main substrates (26).

1.1.3 Oxidative phosphorylation

As its name suggests, oxidative phosphorylation describes ATP regeneration from ADP linked to O₂ consumption (Figure 3). Energy from this system is generated by anaerobic glycolysis and the tricarboxylic acid (TCA) (or Krebs) cycle (Figure 2) (27). Briefly, acetyl-coA, a derivative of pyruvate, is catalyzed to produce H⁺ (27). The electron transport chain (ETC) pumps H⁺ across the inner mitochondrial membrane, creating an electrochemical gradient (27). The resulting protonmotive force drives the phosphorylation of ADP, where O₂ is the final electron acceptor, and H₂O is generated (27). Therefore, O₂ availability and oxidative phosphorylation are intimately linked. At

the onset of exercise, oxidative phosphorylation is delayed despite the rapid increase in PO. During this time, anaerobic systems, especially the ATP-PCr and anaerobic glycolysis systems, supply the energy demand of the exercise (3). It has been demonstrated that the attenuated increase in [ADP] from the PCr system stalls the activation of aerobic ATP generation (28). The slow correction of $\dot{V}O_2$ has been termed the “slow component” and is related to the gradual engagement of the aerobic systems (11). After a few minutes of exercise in our study, oxidative phosphorylation was the major energy ATP regeneration system, which is supported by increasing $\dot{V}O_2$.

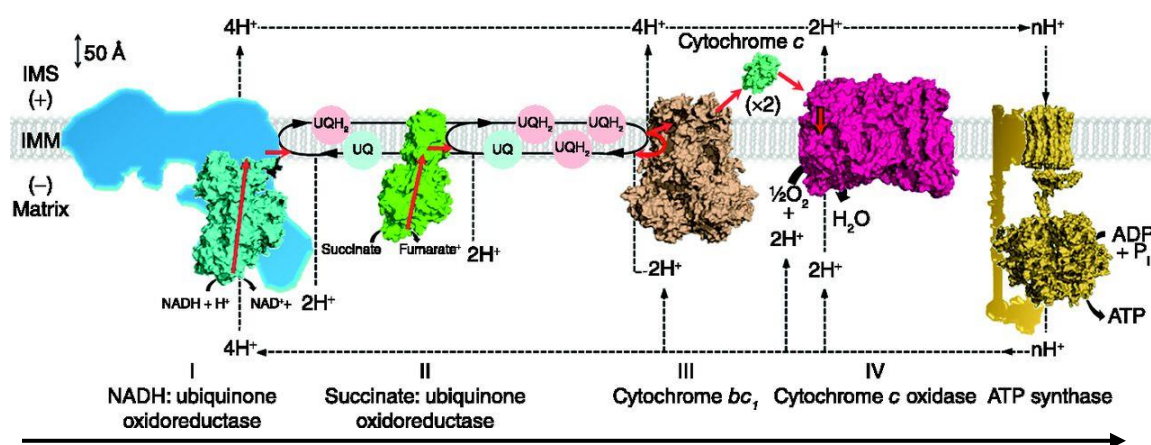


Figure 3. Illustration of the electron transport (respiratory) chain. Electrons are passed through four cytochromes to create an electrochemical H⁺ gradient. The resulting energy drives the generation of ATP by ATP synthase (29).

1.2 « Lactate Threshold »

As PO is increased incrementally from rest, relative energy contributions change from almost purely aerobic to a greater reliance on the anaerobic energy system. Anaerobic glycolytic phosphorylation produces La⁻ and H⁺, which, when efflux is in excess of La⁻ metabolism in the muscle, are released into the blood to be metabolized elsewhere (30). The lactate threshold (LT) describes the PO at which an individual’s blood [La⁻] increases in a non-linear fashion to linear increases in PO. Since La⁻ oxidation is inhibited by O₂ unavailability, this inflection may represent a shift to anaerobic metabolism. The majority of blood H⁺ associated with La⁻ production is buffered by the carbonic anhydrase reaction to produce H₂O and CO₂ (30) (Figure 4). This was historically referred to as “non-metabolic” CO₂ to differentiate it from the “metabolic” CO₂ produced

threshold) defines the PO at which an individual's ventilation (\dot{V}_E) increases beyond the previously observed isocapnic period, during which P_{CO_2} is unchanging and now begins to increase, consequent to $\dot{V}CO_2$ increasing in a non-linear fashion (39). Maintaining a low P_aCO_2 also drives the buffering reaction towards additional CO_2 production and elimination through increased ventilation, due to the law of mass action. However, this model has been contested recently; an alternate explanation for the rise in \dot{V}_E was suggested to be proportional to central nervous activity associated with motor control (40). Instead, it has been suggested that only the tidal volume is impacted by metabolic acidosis and hypercapnia (41), whereas frequency of breathing is modulated with respect to the motor stimulus (40). We expected to observe increased $\dot{V}CO_2$ and end-tidal partial pressures of CO_2 ($P_{ET}CO_2$) in the BH condition of the present study compared to free breathing.

1.4 « Exercise Intensity Domains »

During a ramp incremental (RI) exercise test, there are specific and differential gas exchange and metabolic responses termed exercise intensity domains. There are two similar models that describe these domains, which disagree on the nomenclature of very heavy versus severe exercise.

1.4.1 Moderate-intensity exercise

At POs below the LT, an individual's blood $[La^-]$ and $[H^+]$ production increases linearly with PO (42). Also, $\dot{V}CO_2$ increases steadily with the $\dot{V}O_2$ demands of exercise, which corresponds with exercise under the GET. When exercise is performed at a PO that is insufficient to invoke an inflection of $[La^-]$ and $\dot{V}CO_2/\dot{V}O_2$ common to LT and GET, respectively, it is categorized in the moderate exercise domain (Figure 5). Moderate-intensity exercise is characterized by only oxidative phosphorylation for ATP regeneration. Exercise in this domain can be sustained for prolonged periods (43). The exercise duration is limited by the depletion of intracellular glycogen due to the absence of muscle fatigue (44).

1.4.2 Heavy-intensity exercise

Once disproportionate measurements of blood $[La^-]$ and $\dot{V}CO_2/\dot{V}O_2$ are observed above the ongoing PO increments, then the LT and GET, respectively, have been surpassed. At this PO, this exercise intensity is now classified as heavy-intensity (32, 45) (Figure 5). It has been suggested that one important qualification for exercise in the heavy-intensity is the eventual attainment of a “steady-state” $\dot{V}O_2$ (46). Critical power defines the PO limit at which a steady-state $\dot{V}O_2$ can be reached (47). Above critical power, $\dot{V}O_2$ asymptotically approaches $\dot{V}O_{2max}$, and exercise cannot be sustained indefinitely (32). Similarly, the maximal lactate steady state delineates a similar $\dot{V}O_2$ plateau accompanied by stabilization of blood $[La^-]$. Recent studies have suggested that the maximal lactate steady state may represent a more accurate upper limit for heavy-intensity exercise (48). However, it has also been experimentally demonstrated that the critical power, maximal lactate steady state and respiratory compensation point occur at the same $\dot{V}O_2$, suggesting that they represent similar physiological phenomena (32, 49). Recently, Ozkaya, Balci (50) have suggested a fourth threshold, the critical threshold, that may delineate the heavy-intensity exercise boundary most accurately. The critical threshold is defined as the greatest exercise intensity where steady state $\dot{V}O_2$ is reached under 95% of $\dot{V}O_{2max}$ (50). Nevertheless, these boundaries represent important physiological boundaries that are useful in understanding their effects on maintaining a particular PO during competition or training. Despite the attainment of a steady state, heavy-intensity exercise does provoke muscle fatigue because of the delayed oxidative phosphorylation at the onset of exercise associated with the slow component and the acceleration of fatigue related glycogen depletion (43).

1.4.3 Severe-intensity exercise

The lower boundary of severe exercise is the PO that corresponds with MLSS (Figure 5). According to its definition, exercise at this intensity can only be sustained for a finite duration. Moreover, severe exercise is categorized by the eventual attainment of the individual's $\dot{V}O_{2max}$. This exercise is often terminated due to muscle fatigue associated

with the accumulation of muscle (e.g., La^-) and blood metabolites (e.g., H^+ ; acidosis) (51).

1.4.4 Extreme-intensity exercise

The extreme intensity domain occurs when exercising at a PO above MLSS, but failing to reach $\dot{V}\text{O}_{2\text{max}}$ because the test was terminated prematurely (52) (Figure 5). The categorization of extreme exercise is still unclear in the literature (32); however, there has been increased support for its existence recently (50, 53). This form of exercise can only be sustained for a short duration.

1.4.5 Intensity domains during continuous exercise

In the current study, participants performed four additional exercise transitions each from rest to a continuous intensity (constant-load, CONLD). At the beginning of the exercise transition, there was a delay in O_2 delivery to active muscles despite the increased PO (28). This physiological time delay created a mismatch compared to the O_2 demand of the active muscles (54). Therefore, a greater reliance on anaerobic energy systems was required to fulfill the energy requirements of a high-intensity PO compared to low intensities (11). As exercise continues, $\dot{V}\text{O}_2$ approached a steady state, depending on the intensity of the exercise. However, if the exercise was categorized as severe or extreme, a steady state will not be reached, and exercise will eventually be terminated.

1.4.6 Exercise prescription

Keir, Paterson (32) explained that it is wrong to assume that prescribing exercise by relative $\dot{V}\text{O}_2$ (i.e., 50% of $\dot{V}\text{O}_{2\text{max}}$) above LT elicits identical $\dot{V}\text{O}_2$ for two individuals. Instead, the “delta approach”, which was implemented in the present study, is a method of exercise prescription that calculates a percentage difference between an individual’s $\dot{V}\text{O}_2$ at LT and $\dot{V}\text{O}_{2\text{max}}$ (32). This method may help to prescribe exercise consistently at heavy compared to severe intensities, where steady state $\dot{V}\text{O}_2$ can be reached (32, 43). At the onset of exercise, the light workload allows for a gradual increase in cardiac output (CO) and $\dot{V}\text{O}_2$. Therefore, ATP is regenerated almost exclusively from oxidative

phosphorylation. The domains of exercise will be delineated as they pertain to the increasing PO of the RI test.

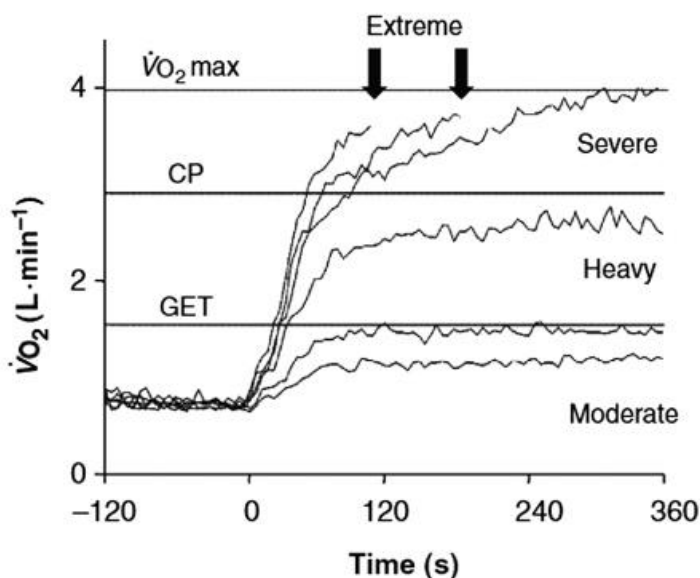


Figure 5. $\dot{V}O_2$ profiles over time for the moderate, heavy, severe, and extreme exercise intensity domains (55). GET, gas exchange threshold; CP, critical power; $\dot{V}O_{2max}$, maximal oxygen consumption; $\dot{V}O_2$, oxygen consumption.

1.5 « Apnea (Breath Holding) and Hypoxia »

Apnea and hypoxia are both conditions that restrict eventual O_2 provision to the body. However, apnea is characterized by the mechanical cessation of breathing, provoking a lack of O_2 supply to tissues that is referred to as hypoxia. Whereas hypoxia may occur without apnea under external hypoxic conditions if the fraction of O_2 in the inspired gas ($F_I O_2$) is below the standard ambient amount ($F_I O_2 \sim 20.93\%$). Longer duration apneas (40 s to 2 min) have been shown to reduce pulmonary $\dot{V}O_2$ during rest (56) and during moderate-intensity exercise (57) due to the reduction of pulmonary gas transport, as reflected by the low alveolar O_2 ($P_A O_2$) (58). However, a paradoxical decreased heart rate (59), and increased arterial Hb O_2 saturation ($S_a O_2$) (59, 60) and vasoconstriction (61) has also been observed that may further limit O_2 availability to working muscles during apnea. This response is an O_2 preservation mechanism has been termed the “human diving response”, as it was first described in elite deep diving athletes (62-64). The bradycardia and vasoconstriction responses are augmented if the BHs are performed with

the face submerged underwater (56, 59, 60). Under BH conditions, it has been suggested that a decrease in CO (59) and peripheral perfusion (61) redistributes blood flow and O₂ towards vital organs (65, 66). Moreover, the attenuated provision of O₂ to working muscles facilitates an increased reliance on anaerobic energy systems (63, 67).

During severe hypoxia, splenic contraction releases an increased amount of red blood cells, which increases hematocrit to increase O₂ carrying capacity (68). This release of Hb from the spleen is associated with low SaO₂, and provokes an increase in total Hb concentration (69). Exercising at high altitudes (hypoxic conditions) augments erythrocyte release (70); therefore, a similar response may occur under hypoxic conditions from BHing, as in the present study. Notably, this response has been observed during repeated BHs (71), which also suggests that splenic contractions are relevant in our study.

Hoffmann, Smerecnik (72) contrasted the deep diving response between apneic and hypoxic conditions during exercise. Participants were instructed to rebreathe (i.e., continuously inspire the exhaled gas) to simulate hypoxia without apnea, since the O₂ in the exhaled gas would be depleted steadily. They found that heart rate and arterial blood pressure did not decrease in the rebreathing condition, suggesting that breathing cessation is necessary to induce the diving response (72, 73). In the present study, participants will perform periods of BHing and regulated breathing during heavy-intensity exercise. If these apneic intervals provoke a diving response, including unchanged SaO₂ and lower $\dot{V}O_2$, it will hinder the ability for the participants to sustain the muscular work. Anaerobic energy sources may play a larger role to maintain ATP regeneration during the ensuing muscle hypoxia under these intermittent apnea conditions.

1.6 « Intermittent Exercise »

Intermittent exercise refers to an exercise bout that is performed under variable intensities throughout. There are two major forms of intermittent exercise: work-rest intervals and work-work intervals, where the intensities of the two work periods are different. The former method involves the repetition of an interval of relatively high-intensity work

followed by a passive recovery period. Belfry, Raymer (5) investigated the effects of a 10 s work period followed by 5 s of recovery (INT) on anaerobic energy system contributions compared to a CONLD protocol. They found that the intramuscular $[H^+]$ was similar during the rest periods of INT compared to the same period during CONLD. The authors showed that increased glycolytic activity during the rest periods contributed to greater PCr resynthesis catalyzed by creatine kinase, as reflected by the unchanged $[H^+]$, which would have occurred had the anaerobic glycolytic energy system been activated (5). Moreover, $[PCr]$ was maintained throughout the intermittent exercise trials, but decreased during CONLD, suggesting an increased reliance on PCr hydrolysis during the work periods of intermittent exercise (5), which has been observed elsewhere (6). Zafeiridis, Kounoupis (74) investigated the muscle oxygenation differences between continuous, short interval (30 s passive recovery) and long interval (2 min passive recovery) exercise protocols. They found no difference in $\Delta[HHb]$ or $\Delta[HHb]/\dot{V}O_2$ between protocols, which reflects similar oxygenation and O_2 provision compared to utilization (74). However, all three protocols observed different averages of $\dot{V}O_2$, which may have altered the muscle oxygenation response.

The other form of intermittent exercise, work-work intervals, incorporates work at two different intensities intermittently. This method of exercise may also be referred to as Fartlek training. Belfry, Paterson (75) investigated the muscle deoxygenation profiles of two intermittent protocols compared to CONLD. Participants were prescribed 10 s work intervals at 50% of the difference between their LT and $\dot{V}O_{2max}$ ($\Delta 50\%$), followed by 5 s of active recovery at 20 W (INT1). In another intermittent protocol (INT2), the 10 s work periods were also performed at $\Delta 50\%$, but the 5 s recovery periods were set at the PO corresponding to 50% of the difference between the participant's $\dot{V}O_2$ at 20W and their LT. CONLD was performed at $\Delta 50\%$. The authors found that ΔHHb and $\Delta HHb/\dot{V}O_2$ were decreased during the 10 s work periods in INT1 and INT2 compared to CONLD. This indicated a greater matching of O_2 delivery to O_2 utilization in the intermittent protocols from an improvement in local blood delivery (75). Furthermore, it was suggested that the higher-intensity recovery periods of INT2 imposed an improved distribution of blood flow during the work intervals compared to INT1, which is reflected by the lower $\Delta HHb/\dot{V}O_2$. They proposed that greater local vasodilation from increased

muscle pump activity and other stimuli during the recovery periods of INT1 and INT2 improves muscle blood perfusion during subsequent work periods.

Furthermore, repeated sprint training is a type of intermittent exercise where supramaximal-intensity exercise is performed periodically. Buchheit, Cormie (76) compared passive and active recovery intervals following 5 s sprint intervals. They found that HHb was lower during the passive recovery periods, which reflected improved blood delivery to active muscle to match O₂ demands. This culminated in greater sprint performance in the passive recovery condition (76). In our study, participants will perform two conditions of work-work intermittent exercise (FLK and FLK-BH), with the shorter interval (5 s) corresponding to a greater PO ($\Delta 50\%$ vs sprint) than the longer interval (25 s).

1.7 « Integrated Response »

It is recommended that competitive swimmers perform underwater kicking after the “flip turn” at maximal velocities to maintain their momentum during resurfacing (77). Thus, to simulate this maximal effort, the present study observed the effects of short (5 s) breath holds during a peak PO (PPO) sprint, as previously investigated (2). The combination of hypoxia and high-intensity exercise mitigates $\dot{V}O_2$ (78, 79) and increases anaerobic metabolism contributions (79). No detriments in muscle blood flow or muscle oxygenation were found when hypoxia was imposed by a low F_IO₂ (78). Decreased breath frequency, such as in the present study, impairs pulmonary gas exchange, which is reflected by a greater difference in the ratio of alveolar to arterial P_{O₂} (A-a ΔO_2) (80). This is because lower breath frequency prevents hyperventilation, which is a major mechanism to attenuate increases in A-a ΔO_2 (81). Correspondingly, lower end tidal P_{O₂} (P_{ET}O₂) and S_aO₂, and higher end tidal CO₂ (P_{ET}CO₂) were observed (82). There is a correlation between P_{ET}O₂ and P_{ET}CO₂ and P_aO₂ and P_aCO₂, respectively, which is modulated by a lower ratio of dead space (V_D) to V_T (V_D-V_T) (83). Since V_D is lower after BHing (84, 85), this may improve the accuracy to which end tidal measurements predict arterial pressures. This is supported by similarly decreased P_aO₂ (hypoxemia) and increased P_aCO₂ during cycling (80) and arm ergometer exercise (86) with reduced f_B. Incorporating periodic BHs during heavy-

intensity exercise was speculated to initiate the diving response, which exacerbates muscle hypoxia and the inhibition of oxidative phosphorylation (2). The transient increases in $\Delta[\text{HHb}]/\dot{V}\text{O}_2$ during the 5 s apneic periods are indicative of the mismatch between O_2 provision and demand imposed by the breathing strategy during exercise (2). This lack of O_2 is reflected by a similar reduction in $\dot{V}\text{O}_2$ (82) compared to during isocapnic hypoxia.

In the present study, $\Delta 50\%$ was chosen as a PO that could be finished with some duress. However, pilot work demonstrated that hypoxia from BHing would accelerate the onset of muscle fatigue and exercise termination (87). Since fatigue may be dependent on a concomitant decrease in S_aO_2 , one may suggest that the O_2 -preserving effects of the diving response, including maintaining S_aO_2 (61), may delay peripheral fatigue (88). Indeed, it has been suggested that when a BH is performed with high lung volume (i.e., after an inspiration), there is some continuous pulmonary gas exchange that mitigates hypoxemia (80). However, it is important to also consider that S_aO_2 was maintained by the inhalation of a hyperoxic mixture in Romer, Haverkamp (88) instead of from BHing (61). Less breathing opportunities could induce hypoxemia (80) and lead to fatigue (88). Therefore, the present study aimed to elucidate the combined effects of BHing and heavy-intensity exercise on these gas exchange and muscle oxygenation variables.

1.8 « Breath by Breath Gas Measurement »

$P_{\text{ET}}\text{O}_2$ and $P_{\text{ET}}\text{CO}_2$ were measured in expired gases at the mouth by a mass spectrometer. During a BH, they were determined by the differences between P_{O_2} and P_{CO_2} in inspired air before the maneuver and expired air after. \dot{V}_E was determined by the flow rate of each breath proportional to the rotation of a bidirectional turbine. The difference between inspired and expired O_2 and CO_2 gas concentrations, together with the flow rate, estimated the volume of O_2 consumption and CO_2 elimination, respectively, from each breath. By incorporating f_B , $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ were calculated.

1.9 « Near Infrared Spectrometry (NIRS) to Measure Regional Muscle Oxygenation »

NIRS is a non-invasive measurement tool used to assess muscle tissue oxygenation by O_2 offloading from Hb (2, 78, 89-91). When the probe is placed on the muscle under investigation, local deoxyhemoglobin (HHb + Mb), oxyhemoglobin (HbO + Mb), total hemoglobin (Hb_{tot}) and Hb O_2 saturation ($S_{at}O_2$) can be continuously measured. Previous literature explains the theory for the data collected by a NIRS device (90, 92). Briefly, the apparatus contains laser diodes that emit light with wavelengths near the infrared region (690 and 828 nm) at a high frequency (110 MHz). This light propagates through skin and adipose tissue, and is dissipated in tissues (90). One of the major mechanisms by which this light is eliminated is O_2 -dependent absorption by Hb (90). Therefore, the absorbance of infrared light may estimate the tissue concentrations of the two important conformers of Hb: [HHb] (690 nm light) and [HbO] (828 nm light) (90, 93). As per the Bohr effect, in acidic or high P_{CO_2} environments, the affinity of HbO to its bound O_2 decreases and causes greater offloading of O_2 to the blood (94). So, during heavy-intensity exercise, metabolic acidosis and hypercapnia permit the diffusion of O_2 into muscle tissue. The corresponding oxygenation effect can be quantified by [HbO] and [HHb] if a NIRS device is securely placed on the working muscles of an exercising human (91, 95).

Light scattering and “geometric factors” (e.g., adipose tissue thickness) decrease the signal-to-noise ratio (SNR) of HbO and HHb (90, 96). Edwards, Richardson (93) suggested that these factors can be considered constant for an individual; the change in absorbance compared to a baseline average over the exercise transition reflects an equal change in the concentrations of HHb ($\Delta[HHb]$) and HbO ($\Delta[HbO]$). Conventionally, $\Delta[HHb]$ has been used as a measure of muscle deoxygenation instead of $\Delta[HbO]$ because the latter may be affected by changes in perfusion under the probe (e.g., skin blood flow, muscle blood flow) (91, 97). The absorbance spectra of HHb and HbO are similar to another chromophore found in the muscle, myoglobin (Mb), which could also decrease the SNR of [HHb] (98). Although research has remained unclear about the significance of

Mb to the signal's strength (99, 100), NIRS remains an accurate, inexpensive and reliable method to determine Hb oxygenation noninvasively (98).

Murias, Spencer (54) and others (6, 101, 102) have suggested that high $\Delta[\text{HHb}]/\dot{V}\text{O}_2$ indicates an adjustment of O_2 delivery to the increasing O_2 demand by offloading additional O_2 from Hb. This Bohr effect is a response to the transient hypoxemia and associated metabolic acidosis experienced in the vasculature (103). It has been suggested that the $\Delta[\text{HHb}]$ breakpoint coincides with increased ventilatory buffering associated with the RCP (104), and that this relationship is preserved in hypoxic conditions (105). This may reflect a concerted hemodynamic and pulmonary effort to delay tissue hypoxia and metabolic acidosis during exercise beyond the heavy-intensity exercise domain. However, as previously mentioned, the breathing restrictions of the present study limit ventilatory buffering.

$[\text{Hb}_{\text{tot}}]$ is the sum of $[\text{HHb}]$ and $[\text{HbO}]$. In the present study, $\Delta[\text{Hb}_{\text{tot}}]$ was derived from $\Delta[\text{HHb}]$ and $\Delta[\text{HbO}]$, measured by NIRS. It has been shown that $\Delta[\text{Hb}_{\text{tot}}]$ can reliably estimate muscle blood flow during isometric knee extension exercise (106, 107). Therefore, in the present study, $\Delta[\text{Hb}_{\text{tot}}]$ was measured to assess differences in perfusion between exercise protocols. $S_{\text{at}}\text{O}_2$, also referred to as the tissue saturation index (TSI), represents the ratio of $[\text{HbO}]$ to $[\text{Hb}_{\text{tot}}]$, expressed as a percentage. Because of its calculation, it suffers from the same perfusion-based caveats as $\Delta[\text{HbO}]$, and therefore is used less as a measure of muscle oxygenation than $\Delta[\text{HHb}]$ (97).

1.10 « Purpose of the Study »

In the present study, short (5 s) BHs and sprints were performed individually and together every 30 s during high-intensity ($\Delta 50\%$ GET and $\dot{V}\text{O}_{2\text{max}}$) (CONLD-BH) and sprint exercise (FLK-BH). Although others utilizing a similar breathing pattern did not evoke a diving response during exercise (2), it was suggested that the post-apneic increases in \dot{V}_E may have accounted for the decreases in $\dot{V}\text{O}_2$ during apnea (2). Thus, it is suggested that the further limited breathing opportunities of the breathing pattern common to front crawl observed in the present study would elicit an O_2 preservation response. We investigated

muscular $\Delta[\text{HHb}]$ and $\Delta[\text{Hb}_{\text{tot}}]$ to determine the relative contributions of muscle oxygenation and perfusion, respectively, on $\dot{V}\text{O}_2$. We expected $\dot{V}\text{O}_2$ to decrease because of insufficient O_2 provision to working muscles related to a decrease in muscle oxygenation ($\Delta[\text{HHb}]$), blood perfusion ($\Delta[\text{Hb}_{\text{tot}}]$), or both.

1.11 « References »

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Chapter 2

2 « The Effect of Breathing Patterns Common to Competitive Swimming on Gas Exchange and Muscle Deoxygenation During Heavy-Intensity Fartlek Exercise »

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2.1 « Introduction »

Competitive swimming requires the performance of high-intensity work while performing regular periods of apnea. For example, the swimming “flip turn” and push off, facilitating a change in direction at the end of the pool, is a maneuver that requires high power output (PO) of the lower extremities (kicking) combined with apnea. International swimming rules stipulate that the swimmers must surface after covering a maximum of 15 meters from the underwater kicking phase. This underwater kicking phase endures for approximately five seconds. Whereas the backstroke affords swimmers to breathe freely during swimming as their face is not underwater, the breaths during the front crawl, performed in the prone position, is confined to the body’s rhythmical rotation along the sagittal plane in the position to when the face is exposed to the air once every stroke cycle. This imposes a regulated breathing paradigm dictated by the specific characteristics of this swimming stroke.

Previous work by Lim, Kim (1) simulated the cardiorespiratory response of the lower extremities to multiple laps of backstroke swimming by repeating cycles of 25 s of free breathing, the approximate duration of swimming one length of a 50 m pool, with 5 s of breath holding (BH) as are experienced during the aforementioned turn and push-off phases. However, since these trials were performed on a cycle ergometer on land, as opposed to in water with facial submersion, their observations only provided a cursory understanding of the physiological effects of the ventilatory techniques that may be common to swimmers. Some swimmers, with better underwater hydrodynamics, may choose to perform a 5 s sprint kick, in combination with the BH, with the hope that the

added swimming speed will offset any negative physiological effects of the sprint in the latter stages of the race. These apneic exercise interventions were separated into four six-min bouts on a cycle ergometer performed at an intensity corresponding to a PO of 50% of the difference between the individual's lactate threshold (LT) and maximal oxygen consumption ($\dot{V}O_{2\max}$) ($\Delta 50\%$): 1) constant-load (CONLD), 2) CONLD with 5 s BHs every 25 s (CONLD-BH), 3) fartlek (25 s at $\Delta 50\%$ and a 5 s sprint) (FLK), and 4) FLK with 5 s BHs every 25 s (FLK-BH). The addition of a BH, which reduced breathing opportunities within CONLD (CONLD-BH), elicited an increase in minute ventilation (\dot{V}_E) during the 25 s free breathing periods, coupled with the elevated deoxyhemoglobin-to- $\dot{V}O_2$ ratio ($[\text{HHb}]/\dot{V}O_2$) during the 5 s BHs. This reflected greater local muscle deoxygenation that supported O_2 utilization and mitigated the repeated hypoxia of the 5 s BH. They suggested that the stimulus for increased ventilation was underpinned by the transient increases in the end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) and decreases in O_2 ($P_{ET}O_2$) during the 5 s BHs, as these factors have been indicated to modulate ventilatory responses (2). Similar to previous apnea studies, the observed decrease in mean total hemoglobin content ($[\text{Hb}_{\text{tot}}]$) and mean concentration of deoxyhemoglobin ($[\text{HHb}]$) during the 5 s apnea period of CONLD-BH compared to CONLD suggests an overall reduction in blood flow and greater reliance on O_2 extraction to support O_2 utilization, reflecting the previously observed apnea driven O_2 conservation response at the level of the muscle (3). The BH did not impose a great enough physiological stress to require a reduction in PO to either the CONLD or FLK condition. However, the added stress of the sprint resulted in decreased $\dot{V}O_2$, despite increases in $[\text{Hb}_{\text{tot}}]$ and $[\text{HHb}]$. The redistribution of blood flow and reduced cardiac output (3) have been replicated before under apneic conditions (4, 5), and are similarly experienced during deep diving (6). Hb provision to the blood has also been observed during these dives as a function of splenic contractions (7), which elicit greater O_2 and CO_2 carrying capacities in blood. This deep diving response does not appear to extend to high-intensity knee extensions (8) or cycling exercise (9) under hypoxic conditions.

Prone swimming strokes impose a regulated breathing paradigm that should abolish the transient increases in \dot{V}_E during free breathing. However, to what extent this is true remains unknown and could be evaluated by imposing a paradigm where regulation of

each individual's frequency of breathing (f_B) and inspiratory tidal volume (VTI) are controlled for each minute of exercise.

Therefore, the purpose of this study was to investigate respiration and muscle deoxygenation under regulated breathing versus free breathing conditions between the aforementioned protocols. We hypothesized that with the reduced breathing opportunities from the regulated ventilation during the 25 s breathing periods: 1) mean $\dot{V}O_2$ would decrease, and mean $\dot{V}CO_2$ would decrease in CONLD-BH compared to CONLD and FLK-BH compared to FLK. Moreover, 2) relative to the conflicting results between CONLD-BH and FLK-BH in Lim, Kim (1), we expected to observe increases in $[Hb_{tot}]$, $[HHb]$ and $[La^-]$ to account for the increased physiological stress of regulated breathing during both conditions.

2.2 « Materials and Methods »

Ten males (mean 23.7 ± 2.5 years) participated in this study after their written informed consent was given. Inclusion criteria were that the participants were healthy and active (i.e., exercising 1-3 times per week). Smokers and individuals who take medication for the cardiopulmonary system were excluded. None of the participants were involved in sports or other recreational activities. They were instructed to adhere to 30 to 60 min of moderate-heavy-intensity resistance training of the upper and lower extremities two to three times each week. All procedures were approved by the Western University Research Ethics Board for Health Sciences Research Involving Human Participants and were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Baseline characteristics for the participants are available in Table 1.

2.2.1 Test conditions

Individual activity levels were maintained throughout the duration of this study. Participants were asked to avoid caffeine for 6 h prior to each test. Five tests were performed on an electronically braked cycle ergometer, each on a separate day with a minimum of 48 hours between tests. Participants wore a nose clip to prevent nose

breathing, and a mouthpiece to facilitate gas exchange analysis and ventilatory measurements.

2.2.1.1 Ramp incremental test (day 1)

Participants were instructed to complete a ramp test to exhaustion on a cycle ergometer. The PO increased by 25 W/min while a cadence of 70 rpm was maintained. Verbal encouragement was given to participants to maximize performance. When participants were unable to cycle above 60 rpm for more than 5 consecutive seconds, the protocol was stopped. This incremental test was used to determine the maximal aerobic capacity ($\dot{V}O_{2\max}$), peak power output (PPO) and the estimated lactate threshold (LT). Above the LT, excess $[H^+]$ derived from the increase in lactate production is buffered by the carbonic anhydrase reaction, which yields greater CO_2 output in relation to O_2 utilization than that observed when exercising below the LT (10). This increased CO_2 results in an increase in \dot{V}_E , which is reflective of the LT. Therefore, this estimated LT was used as a proxy of the actual LT as the exercise intensity at which $\dot{V}CO_2/\dot{V}O_2$ began to increase disproportionately to increases in PO (also referred to as the gas exchange threshold).

2.2.1.2 Constant load exercise (CONLD)

A constant-load step procedure (CONLD) was performed at the PO at 20% of the difference between individual LT and $\dot{V}O_{2\max}$ ($\Delta 20\%$). A 4 min baseline period at 20 W was followed by constant-load cycling at 70 rpm for 6 min while free breathing to stabilize the gas exchange response. Five f_B measurements were recorded at 1 min, 2 min, 3 min, 4 min, and 5 min after the onset of the PO.

2.2.1.3 Constant load with BHs (CONLD-BH)

Another square wave test similar to CONLD was performed by participants at $\Delta 20\%$. Every 25 s from the beginning of the warm-up, participants performed a 5 s BH. A 5 s countdown was given prior to each BH period. During the remaining 25 s of the 30 s cycles, breathing was regulated to CONLD: participants matched their f_B and tidal volume (VT) for each minute of exercise to that achieved during the CONLD condition,

with the guidance of a metronome and feedback from the attending researcher, respectively.

2.2.1.4 Fartlek (FLK)

After the initial 4 min warm-up, participants commenced heavy-intensity work at $\Delta 20\%$ for 6 min with a cadence of 70 rpm. Every 25 s, a 5 s interval at the individual's PPO was performed. Subjects could breathe freely. Like in CONLD, f_B measurements were recorded for each of the first five minutes after the onset of exercise.

2.2.1.5 Fartlek with BHs (FLK-BH)

Lastly, a similar protocol to FLK was performed by participants. 5 s BHs were incorporated every 25 s, so the sprints were performed under apnea. f_B was matched to the minute-by-minute measurements taken in FLK.

The ramp protocol was always performed on day one. Each participant was required to complete CONLD and FLK before CONLD-BH and FLK-BH, respectively to establish the ventilatory thresholds for the BH protocols. Otherwise, each participant was randomly prescribed one of the six possible orders to complete the submaximal exercise conditions (e.g., CONLD, FLK, FLK-BH, CONLD-BH) by an online random sequence generator. Comparisons of the physiological outcomes were performed within each participant's performance between apneic and non-apneic conditions, such that participants acted as their own controls.

2.2.1.6 Experimental considerations

During pilot testing, we noted that no individuals were able to complete the 6 min work at the prescribed $\Delta 50\%$ PO with the periodic BHs and/or sprints. The PO was reduced until all participants were able to complete these 6 min trials. This PO corresponded with a prescribed PO of $\Delta 20\%$.

2.2.2 Measurements

Breath-by-breath gas exchanges and ventilatory rates at the mouth were assessed via a mass spectrometer (Innovision, AMIS 2000, Lindvedvej, Denmark), and are described in detail elsewhere (11). Briefly, flow rates during inspiration and expiration were determined with a low dead space bidirectional turbine (Alpha Technologies, VMM 110) and pneumotach (Hans Rudolph, Model 4813) calibrated with a 3 L syringe. Gas samples at the mouth were analyzed for O₂, CO₂ and N₂ concentrations. Changes in gas concentrations were matched to the corresponding increase or decrease in gas volume. There was a 20 ms interval between collection samples, which were sent electronically to a computer to analyze individual breaths. Each breath started with inspiration and ended with expiration. Therefore, each 5 s BH was recorded as a single breath.

The procedures for near-infrared spectroscopy (NIRS) data collection were similar to those previously described (12). Continuous measurement of the quadriceps was performed with a NIRS device (Oxiplex TS, model 95,205, ISS, Champaign, IL). Laser diodes pulsed quickly (110 MHz) at two different wavelengths near the infrared region (690 and 828 nm). These were connected to a plastic probe that was placed midway between the lateral epicondyle and greater trochanter of the femur on the belly of the vastus lateralis muscle head. An elastic strap secured the device in place. An optically dense, black vinyl sheet was placed over the device to prevent exposure to extraneous light. A tensor bandage was wrapped gently around the participant's leg to secure the NIRS device to the site of interest and further prevent the intrusion of external light into the site of NIRS measurement. Care was taken to ensure that no blood flow occlusion occurred to the leg. Deoxygenated hemoglobin concentration ([HHb]) and oxygenated hemoglobin ([HbO]) were measured, whereas total hemoglobin concentration ([Hb_{tot}]) and tissue oxygen saturation (S_{at}O₂) were derived with this apparatus. [Hb_{tot}] was calculated as the sum of [HHb] and [HbO], and S_{at}O₂ was estimated as the percent of [HbO] to [Hb_{tot}]. To account for individual differences in absolute tissue absorption, [HHb] and [Hb_{tot}] were adjusted to baseline values (Δ [HHb] and Δ [Hb_{tot}], respectively).

Rubbing alcohol was applied to the left index finger of each participant before each test. Blood lactate concentrations ($[La^-]$) were taken three minutes pre- and post-exercise for each test. A lancet (ACCU-CHEK Safe-T-Pro Plus) exposed blood on the finger, which was analyzed by the SensLab GmbH Lactate SCOUT arterialized-capillary lactate analyzer.

2.2.3 Analysis

Gas exchange and NIRS data were cleaned by the removal of aberrant datapoints that were at least 3 SDs from the local mean. The data were interpolated linearly to convert from breath-by-breath to 1 s intervals. Then, datapoints were averaged into 5 s bins. This analysis technique has been described previously (13). $[HHb]$ and $[Hb_{tot}]$ were zeroed with baseline as described earlier. These baselines represented the average of the 60 s before the square-wave change in PO. $\Delta[HHb]/\dot{V}O_2$ was determined as the ratio between the normalized $[HHb]$ to $\dot{V}O_2$. Between-group comparisons on gas exchange and NIRS variables were performed from the start of the square-wave change in PO (the exercise on-transient) to the end of the bout (0 s to 360 s). Within-group comparisons were performed between the first 25 s and the last 5 s of each 30 s cycle for $\dot{V}O_2$ and $\dot{V}CO_2$. Lamarra, Whipp (14) describes this mono-exponential function that models the on-transient $\dot{V}O_2$ curve during a step transition:

$$y(t) = y_{BSL} + A_p (1 - e^{-(t-TD)/\tau}) \quad (1)$$

Here, $y(t)$ represents the $\dot{V}O_2$ as a function of time during the transition to the new PO's steady state. y_{BSL} is the $\dot{V}O_2$ before the transition, A_p is the amplitude (increase above y_{BSL}), t is the dependent time variable, TD is the time delay and τ is the time constant (time that elapses for 63% of the response to occur). The curve was fit to the data by applying the Levenberg-Marquardt algorithm for non-linear least squares analysis (Origin 9.7; OriginLab, Northampton, MA).

2.2.4 Statistics

10 participants were recruited based on a sample size power calculation of the measured $\dot{V}O_{2max}$. It was found that 10 participants were sufficient (80% power) to calculate pre vs

post $\dot{V}O_{2\max}$ ($\alpha=0.05$) to within a SD of $\pm 30\text{mL}$ with the consideration of a 20% drop out rate. Mean analyses from 0 s (start of square-wave change in PO) to 360 s (end of exercise) ($n=10$) of $\dot{V}O_2$, $\dot{V}CO_2$, $P_{ET}O_2$, $P_{ET}CO_2$, \dot{V}_E , f_B , VTI, $\Delta[\text{HHb}]$, $\Delta[\text{Hb}_{\text{tot}}]$, $S_{\text{at}}O_2$ and $\Delta[\text{HHb}]/\dot{V}O_2$ between conditions (CONLD, CONLD-BH, FLK, and FLK-BH) were performed by a one-way repeated measures (1-RM) analysis of variance (ANOVA). $[\text{La}^-]$ was compared between conditions and time (PRE vs POST) by a two-way RM (2-RM) ANOVA. The first 25 s and last 5 s of each apneic cycle were analyzed for $\dot{V}O_2$ and $\dot{V}CO_2$ via a 2-RM ANOVA. Shapiro-Wilk and Brown-Forsythe tests were performed to assess normality and heteroscedasticity of the data, respectively. The 1-RM ANOVA comparisons for $\dot{V}CO_2$, $P_{ET}O_2$, $P_{ET}CO_2$, \dot{V}_E , f_B , VTI, $\Delta[\text{HHb}]$, $\Delta[\text{Hb}_{\text{tot}}]$, $S_{\text{at}}O_2$ and $\Delta[\text{HHb}]/\dot{V}O_2$ were performed by a Friedman RM ANOVA on ranks due to a rejection of either, or both tests, whereas mean $\dot{V}O_2$ was evaluated with a parametric 1-RM ANOVA. Data are reported as mean \pm SD. The statistical significance threshold was $p<0.05$.

2.3 « Results »

Participants successfully matched \dot{V}_E between CONLD and CONLD-BH ($p=0.889$), and FLK and FLK-BH ($p=0.889$) between the onset of the square-wave increase in PO and exercise cessation (Table 2; Figure 6A). This was supported by the sustained mean f_B between CONLD and CONLD-BH ($p=0.72$), and FLK and FLK-BH ($p=0.99$) (Figure 6B), and mean VTI between CONLD and CONLD-BH ($p=0.953$) (Figure 6C). VTI was statistically greater in FLK-BH compared to FLK ($p<0.001$) by $\sim 80\text{mL}/\text{min}$.

2.3.1 Gas exchange variables

Mean $\dot{V}O_2$ from time 0 to 360 s was similar between CONLD and CONLD-BH ($p=0.406$), and between FLK and FLK-BH ($p=0.165$) (Table 3; Figure 7A). $\dot{V}O_2$ was greater in FLK and FLK-BH than in CONLD ($p<0.001$ for both comparisons), and greater in FLK than CONLD-BH ($p<0.001$) over the same time period. Mean $\dot{V}O_2$ for the last 5 s of each 30 s cycle was greater in CONLD than CONLD-BH ($p=0.001$), and greater in FLK than in FLK-BH ($p<0.001$) and CONLD-BH ($p<0.001$) (Table 4). Mean $P_{ET}O_2$ was greater in CONLD than CONLD-BH ($p<0.001$), but was unchanged in FLK

compared to FLK-BH ($p=0.682$) (Table 3; Figure 7C). $P_{ET}O_2$ was lower in CONLD and CONLD-BH than in FLK and FLK-BH ($p<0.001$ for all comparisons) (Table 3; Figure 7C). $P_{ET}CO_2$ was lower in CONLD than in CONLD-BH ($p<0.001$), but was greater in FLK than in FLK-BH ($p<0.001$) (Table 3; Figure 7D). $\dot{V}CO_2$ from 0 s to the end of exercise was similar between CONLD and CONLD-BH ($p=0.914$), and between FLK and FLK-BH ($p=0.641$), but was greater in FLK and FLK-BH than in CONLD and CONLD-BH ($p<0.001$ for all comparisons) (Table 3; Figure 7B).

2.3.2 $\Delta[Hb_{tot}]$, $\Delta[HHb]$, $S_{at}O_2$ and $\Delta[HHb]/\dot{V}O_2$

NIRS-derived normalized total hemoglobin content ($\Delta[Hb_{tot}]$) was greater in CONLD compared to CONLD-BH ($p<0.001$), FLK ($p<0.001$) and FLK-BH ($p<0.001$), as well as in FLK compared to FLK-BH ($p<0.001$) (Table 3; Figure 8C). Deoxygenated hemoglobin normalized to baseline ($\Delta[HHb]$) was greater in CONLD than in CONLD-BH ($p=0.011$) and FLK ($p<0.001$), and also greater in FLK-BH than in the other 3 conditions ($p<0.001$ for all comparisons) from onset to the cessation of exercise (Table 3; Figure 8A). Oxygen saturation ($S_{at}O_2$) was greater in CONLD compared to CONLD-BH ($p<0.001$), FLK ($p<0.001$) and FLK-BH ($p<0.001$), and it was not significantly different in FLK compared to FLK-BH ($p=0.434$) (Table 3; Figure 8D). The ratio of $\Delta[HHb]/\dot{V}O_2$ was similar between CONLD and CONLD-BH ($p=0.086$), but lower in FLK than in FLK-BH ($p<0.001$) (Table 3; Figure 8B).

2.3.3 Lactate ($[La^-]$)

Post-exercise arterialized capillary lactate concentration ($[La^-]$) was unchanged in CONLD compared to CONLD-BH ($p=1.000$), and both were lower compared to FLK and FLK-BH ($p<0.001$ and $p=0.007$, respectively) (Table 3; Figure 9). Post-exercise $[La^-]$ was similar between FLK and FLK-BH ($p=1.000$) (Table 3; Figure 9).

Table 1. Baseline participant characteristics and results from the incremental ramp test (n=10).

Variable	Mean \pm SD
Age	23.7 \pm 2.5
Height (cm)	175 \pm 6
Weight (kg)	78.5 \pm 9.0
$\dot{V}O_{2max}$ (L/min)	3.23 \pm 0.63
$\dot{V}O_2$ at LT (L/min)	1.72 \pm 0.16
PO at LT (W)	155 \pm 23
PO at $\Delta 20\%$ (W)	189 \pm 23
PPO (W)	303 \pm 36

Table 2. Breathing frequency (f_B), inspiratory tidal volume (VTI), and minute ventilation (\dot{V}_E) under each condition. CONLD-BH was matched to CONLD and FLK-BH was matched to FLK. Values are mean \pm SD. ^a Significantly different than CONLD. ^b Significantly different than CONLD-BH. ^c Significantly different than FLK.

Variable	CONLD	CONLD-BH	FLK	FLK-BH
f_B (breaths/min)	22.0 \pm 4.7	21.2 \pm 4.5	25.2 \pm 5.2 ^{a,b}	24.2 \pm 6.0 ^{a,b}
VTI (L)	2.93 \pm 0.21	2.91 \pm 0.27	2.86 \pm 0.30 ^{a,b}	2.94 \pm 0.24 ^{a,c}
\dot{V}_E (L/min)	64.8 \pm 16.9	62.4 \pm 17.5	72.7 \pm 20.0 ^{a,b}	73.1 \pm 22.1 ^{a,b}

Table 3. Mean outcome measures for each of the four conditions: CONLD, CONLD-BH, FLK and FLK-BH from 0 to 360 s. Data are reported as mean \pm SD. ^a Significantly different than CONLD. ^b Significantly different than CONLD-BH. ^c Significantly different than FLK. POST is 3 min post-exercise.

Variable	CONLD	CONLD-BH	FLK	FLK-BH
$\dot{V}O_2$ (L/min)	2.12 \pm 0.35	2.15 \pm 0.42	2.24 \pm 0.40 ^{a,b}	2.20 \pm 0.45 ^a
$\dot{V}CO_2$ (L/min)	2.30 \pm 0.55	2.29 \pm 0.64	2.55 \pm 0.65 ^{a,b}	2.43 \pm 0.70 ^{a,b}
$P_{ET}O_2$ (mmHg)	99.4 \pm 5.3	96.9 \pm 4.9 ^a	101.3 \pm 5.0 ^{a,b}	101.5 \pm 5.4 ^{a,b}
$P_{ET}CO_2$ (mmHg)	45.0 \pm 2.2	46.3 \pm 3.0 ^a	45.1 \pm 2.6 ^b	44.0 \pm 2.6 ^{a,b,c}
$\Delta[Hb_{tot}]$ (μM)	3.3 \pm 1.6	-2.5 \pm 1.2 ^a	2.0 \pm 1.6 ^{a,b}	0.82 \pm 1.4 ^{a,b,c}
$\Delta[HHb]$ (μM)	7.3 \pm 1.8	7.0 \pm 2.0 ^a	6.7 \pm 1.8 ^{a,b}	8.7 \pm 2.4 ^{a,b,c}
$S_{at}O_2$ (%)	62.6 \pm 1.9	59.4 \pm 3.3 ^a	61.2 \pm 2.2 ^{a,b}	61.7 \pm 3.1 ^{a,b}
$\Delta[HHb]/\dot{V}O_2$	3.38 \pm 0.52	3.25 \pm 0.83	2.96 \pm 0.64 ^{a,b}	3.88 \pm 0.98 ^{a,b,c}
POST [La^-] (mM)	9.4 \pm 2.4	10.0 \pm 2.6	11.5 \pm 2.5 ^{a,b}	11.3 \pm 2.8 ^{a,b}

Table 4. Mean outcome measures for both BH conditions (CONLD-BH and FLK-BH) for each 30 s apneic cycle (25 s regulated breathing, 5 s apnea) from 0 to 360 s. Data are reported as mean \pm SD. * 25 s significantly different than 5 s.

Variable	CONLD-BH		FLK-BH	
	25 s	5 s	25 s	5 s
$\dot{V}O_2$ (L/min)	2.19 \pm 0.24	1.93 \pm 0.27 *	2.25 \pm 0.38	1.89 \pm 0.27 *
$\dot{V}CO_2$ (L/min)	2.33 \pm 0.23	2.04 \pm 0.27 *	2.49 \pm 0.34	2.11 \pm 0.22 *
$P_{ET}O_2$ (mmHg)	98.0 \pm 4.4	90.9 \pm 3.7 *	103.0 \pm 4.9	93.5 \pm 4.4 *
$P_{ET}CO_2$ (mmHg)	45.8 \pm 4.1	48.8 \pm 4.1 *	43.4 \pm 4.6	47.2 \pm 4.3 *
$\Delta[Hb]$ (μ M)	7.0 \pm 6.2	7.3 \pm 6.5 *	8.6 \pm 9.6	8.9 \pm 9.8 *

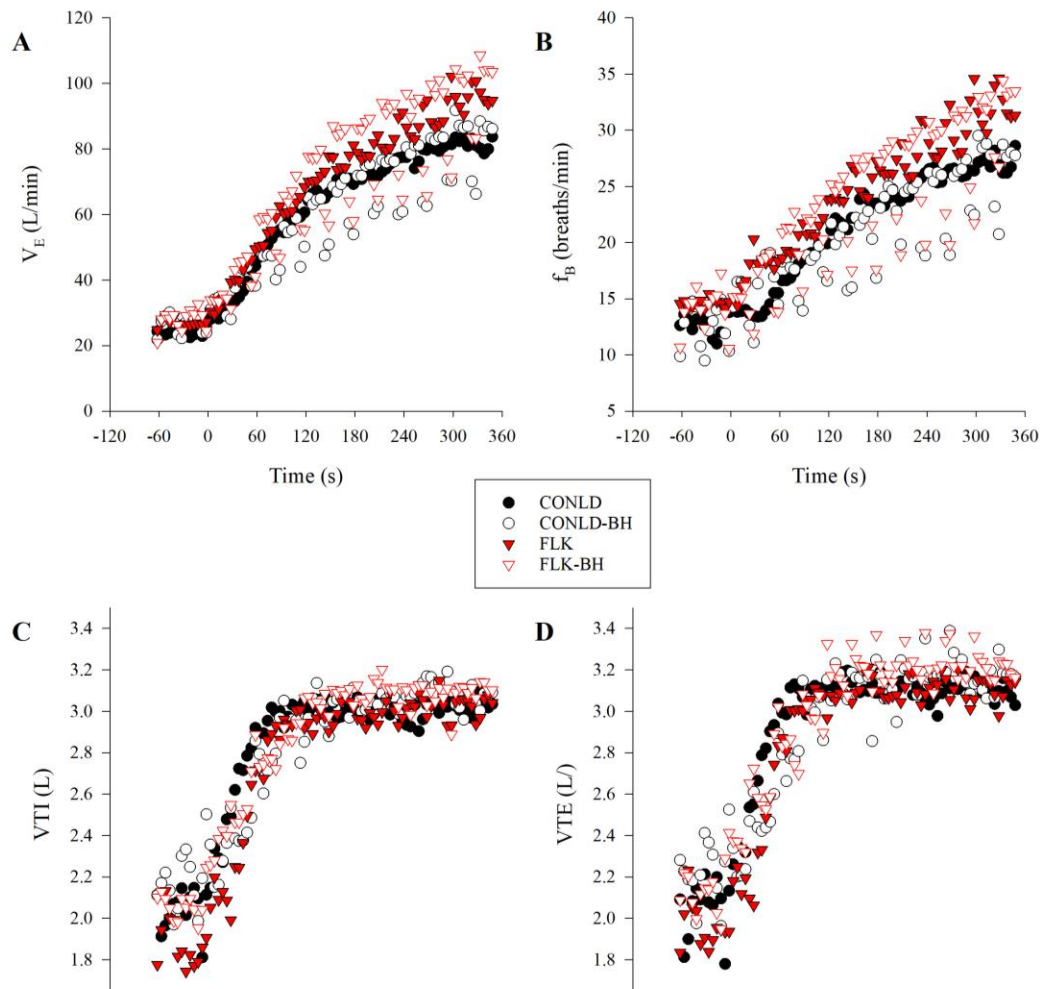


Figure 6. Breathing data during CONLD (filled circles), CONLD-BH (open circles), FLK (filled red triangles) and FLK-BH (open red triangles). (A) Minute ventilation (\dot{V}_E), (B) frequency of breathing (f_B), (C) inspiratory tidal volume (VTI), and (D) expiratory tidal volume (VTE). Time 0 marks the start of the exercise transition. Data were interpolated from breath-by-breath to second-by-second and 5 s averaged for graphical representation.

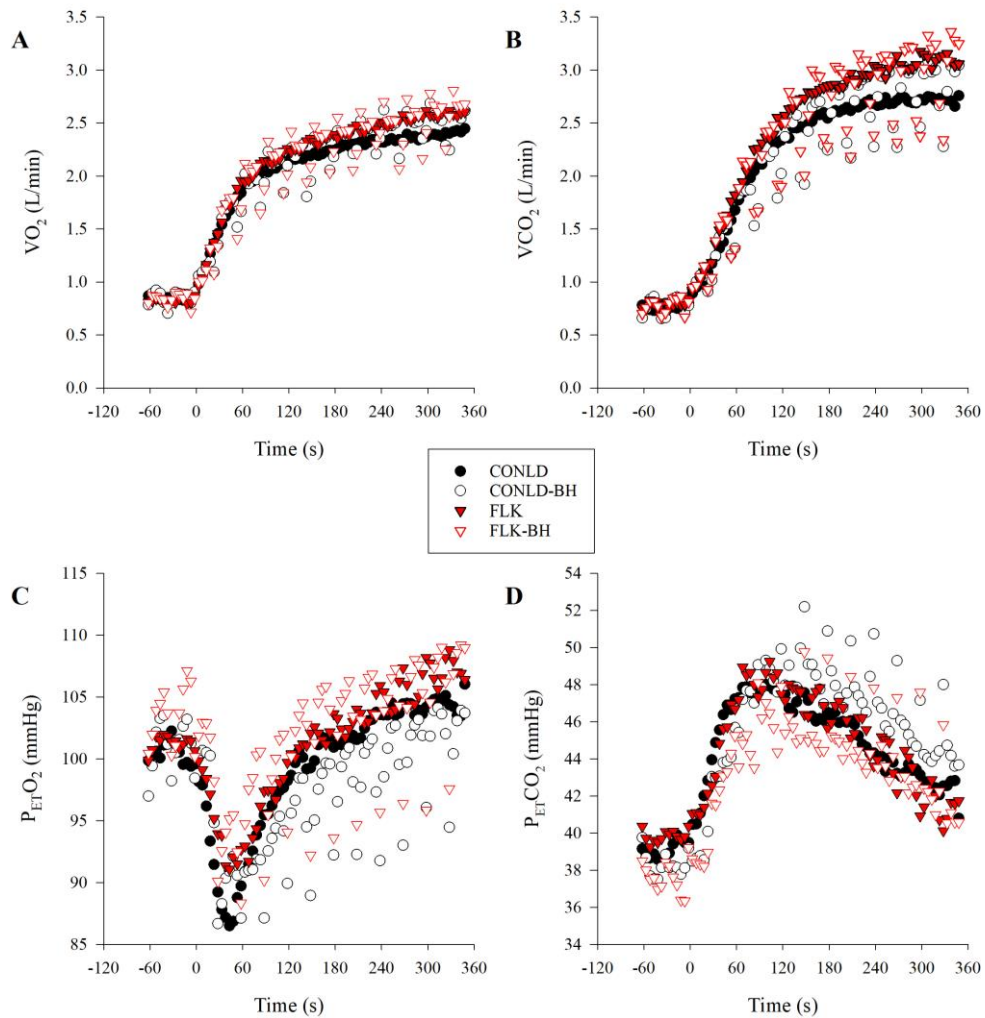


Figure 7. Mean participant respiratory variables during constant $\Delta 20\%$ work (CONLD, filled circles), constant $\Delta 20\%$ work with 5 s BHs every 30 s (CONLD-BH, open circles), $\Delta 20\%$ work with 5 s sprints at peak power output (PPO) every 30 s (FLK, filled red triangles) and $\Delta 20\%$ work with 5 s BHs and sprints at PPO every 30 s (FLK-BH, open red triangles) for 6 min. **(A)** Oxygen consumption ($\dot{V}O_2$), **(B)** carbon dioxide elimination ($\dot{V}CO_2$), **(C)** end-tidal partial pressure of oxygen ($P_{ET}O_2$), and **(D)** end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$). Time 0 marks the start of the exercise transition. Data were interpolated from breath-by-breath to second-by-second and 5 s averaged for graphical representation.

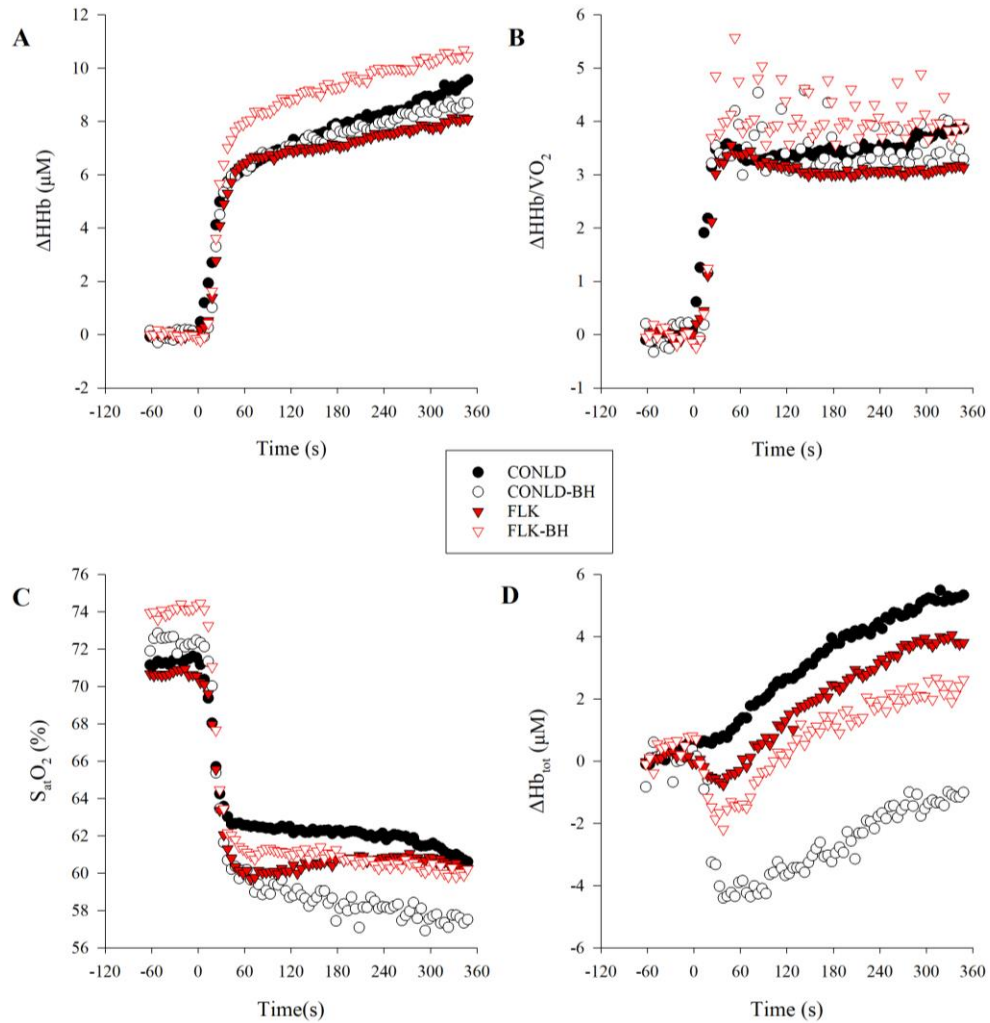


Figure 8. Local muscle deoxygenation variables from near infrared spectrometry (NIRS) data in CONLD (filled circles), CONLD-BH (open circles), FLK (filled red triangles) and FLK-BH (open red triangles). (A) Deoxyhemoglobin concentration normalized to baseline ($\Delta[\text{HHb}]$), (B) ratio of normalized deoxyhemoglobin to $\dot{V}\text{O}_2$ ($\Delta[\text{HHb}]/\dot{V}\text{O}_2$), (C) oxygen saturation of hemoglobin, and (D) total hemoglobin concentration normalized to baseline ($\Delta[\text{Hb}_{\text{tot}}]$). Time 0 marks the start of the exercise transition. Data were interpolated from breath-by-breath to second-by-second and 5 s averaged for graphical representation.

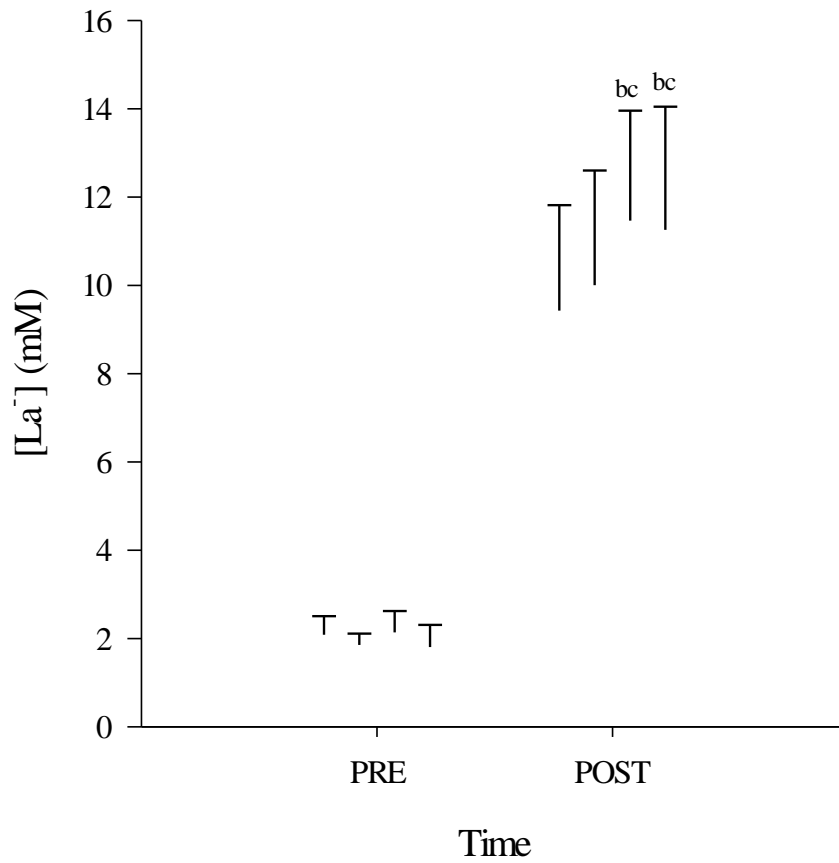


Figure 9. Arterialized capillary lactate concentrations ($[La^-]$) PRE and POST exercise in CONLD (white), CONLD-BH (white with diagonal lines), FLK (gray) and FLK-BH (gray with diagonal lines). All four POST $[La^-]$ were greater than the PRE $[La^-]$. ^a Significantly greater than CONLD. ^b Significantly greater than POST CONLD. ^c Significantly greater than POST CONLD-BH.

2.4 « Discussion »

The goal of this study was to monitor the physiological adjustments to repeated cycles of 5 s BHs followed by 25 s of regulated breathing during CONLD and FLK exercise lasting 6 min. Main findings included: i) unchanged $\dot{V}O_2$ between CONLD and CONLD-BH, and in FLK and FLK-BH, despite the imposed regulatory breathing paradigm; ii) $\Delta[\text{Hb}_{\text{tot}}]$, $\Delta[\text{HHb}]$, and $S_{\text{at}}O_2$ were lower in CONLD-BH compared to CONLD, whereas $\Delta[\text{HHb}]$ and $S_{\text{at}}O_2$ were greater in FLK-BH compared to FLK.

2.4.1 CONLD and CONLD-BH

The CONLD and CONLD-BH PO changes, relative to the previous related research (1) reflect the additions of the regulated breathing to this 25 s breathing: 5 s apnea protocol. The purpose of this modification was to simulate the difference in breathing patterns between swimming backstroke (25 s free breathing) and front crawl (25 s regulated breathing). By instituting these restrictions, participants were forced to perform a much lower-intensity (189 W, $\Delta 20\%$) compared to Lim, Kim (1) (218 W, $\Delta 50\%$, $p=0.027$), despite having similar aerobic fitness ($\dot{V}O_{2\text{max}}$: 3.23 L/min and 3.17 L/min, $p=0.82$, respectively), lactate thresholds ($\dot{V}O_2$ at LT: 1.72 L/min and 1.77 L/min, $p=0.57$, respectively; PO at LT: 155 W and 156 W, respectively), and PPO (303 W and 314 W, $p=0.58$, respectively) to perform the six minute trials. Moreover, this reduced PO of the present study elicited lower mean \dot{V}_E (68 L/min) compared to Lim, Kim (1) (99 L/min). Within the context of this PO adjustment of the present study, $\dot{V}O_2$ was unchanged with the addition of the 5 s apneic periods and matching of f_B and VTI during the 25 s breathing periods (Table 2) of CONLD-BH, demonstrating that any potential increase in O_2 cost derived from the periodic apneas had been met. The data in the present study show that the aerobic metabolic demand (i.e., $\dot{V}O_2$) was supported by the decreased $S_{\text{at}}O_2$ (Table 3, Figure 8C) and increased muscle deoxygenation under the reduced perfusion conditions, as reflected by the much greater decrease of mean $\Delta[\text{Hb}_{\text{tot}}]$ (Table 3, Figure 8D) and unchanged $\Delta[\text{HHb}]$ (Table 3, Figure 8A). A similar reduction in $S_{\text{at}}O_2$ and increase in muscle deoxygenation was observed by Kume, Akahoshi (15) at a PO corresponding to 65% of $\dot{V}O_{2\text{max}}$ interspersed with 4 s exhalations followed by a maximal

inhalation, simulating hypoventilation. Furthermore, previous work by Hoffmann, Smerecnik (4) under apneic and rebreathing exercise conditions during higher-intensity exercise, which generated similar hypoxia (decreased $P_{ET}O_2$ in rebreathing), demonstrated greater mean arterial pressure and lower heart rate in the apneic compared to the rebreathing condition demonstrating that only the apneic state elicited the observed O_2 conservation or diving response that is associated with the decreased intramuscular blood flow. They concluded, that the mechanical cessation of breathing was the key stimulus to this drop in perfusion, which has been corroborated by similar protocols comparing rebreathing with apnea (16). The data of the current study confirms a similar response under the combination of the regulated breathing and BH paradigm. Moreover, the observed decrease in $\Delta[Hb_{tot}]$ shown in the present study was not observed during hypoxic exercise (~12% fraction of oxygen in inspired air [$F_{I}O_2$]) performed at low-moderate (single-leg knee extensions) (17) and supra-lactate threshold intensities (18), suggesting, again, that the reduced blood flow in the present study was apnea related. Furthermore, earlier research by Lim, Kim (1), within a comparable protocol as the present study, and Kume, Akahoshi (19) performing intermittent apneas during continuous exercise at a comparable intensity, identified similar temporal reductions in $\Delta[Hb_{tot}]$ resolved by similar increases in muscle deoxygenation. This decrease was observed despite previous suggestions that $\Delta[Hb_{tot}]$ is increased during deep diving via splenic contractions (7).

The drop in $P_{ET}O_2$ (Table 4; Figure 7C) and increased $\dot{V}O_2$ (Table 4; Figure 7A) observed immediately post-apnea in the present study reflect continued alveolar to capillary O_2 diffusion facilitating the unchanged $\dot{V}O_2$. This response has also been observed under 20 s apneas (20 s) (5) but also reflect the actual arterial O_2 (P_aO_2) under these transient apneic conditions (20).

The maintenance of mean $\dot{V}O_2$ between CONLD and CONLD-BH after the exercise on-transient response was not only supported by the microvasculature hemodynamic changes outlined earlier, but also by the increased $\dot{V}O_2$ during the 25 s of regulated breathing (Table 4; Figure 7A) and the reduced $P_{ET}O_2$ immediately post-BH (Table 4; Figure 7C) suggested earlier (21). Specifically, Woorons, Mollard (22) found that intermittent apneas

performed at a high pulmonary volume (i.e., near total lung capacity) during higher-intensity exercise ($\sim 70\% \dot{V}O_{2\max}$), similar to the current study, maintained pulmonary arterial $S_{at}O_2$.

The unchanged mean $\dot{V}CO_2$, between CONLD and CONLD-BH, suggests that the H^+ buffering, associated with the lactate production of anaerobic glycolysis has been reflected in the observed increase of $P_{ET}CO_2$ and that the BH did not impose a greater anaerobic glycolytic contribution. The unchanged post-exercise $[La^-]$ between CONLD and CONLD-BH trials is in contrast to other studies that have reported increased $[La^-]$ accumulation during incremental (23) (50W + 12.5W/min to exhaustion) and intermittent (1) exercise under hypoxic or apneic conditions, respectively. However, these studies were performed at much higher supra-lactate threshold PO, which would elicit much greater blood $[La^-]$.

From a swimming front crawl perspective, at this $\Delta 20\%$ PO intensity, the addition of the regulated breathing paradigm and the 5 s BH, while maintaining a similar intensity of kicking, may be performed without negative physiological consequences. Notably, this is within the context of the legs only paradigm of this experiment as opposed to the simultaneous arm and leg action that is performed after the underwater portion of the swim.

2.4.2 FLK and FLK-BH

The FLK condition mimics the strategies of those swimmers who believe that sprint kicking during the underwater portion after the turn, coupled with their own superior hydrodynamics compared to their opponents will give them a competitive advantage. In contrast to Lim, Kim (1), utilising a similar protocol, albeit at much higher PO, our results showed that implementing the intermittent 5 s periods of apnea to FLK (FLK-BH) did not affect mean $\dot{V}O_2$. Similar to CONLD-BH and CONLD, f_B and VTI were also regulated in FLK-BH to FLK, however, although VTI was statistically greater in FLK-BH (Table 2), the unchanged \dot{V}_E suggested that this $\sim 80\text{mL}/\text{min}$ change in VTI ($\Delta 1.6\%$ over the 6 min) had no physiological effects. It was notable that VTI was lower in FLK compared to CONLD (2.86 ± 0.30 L and 2.93 ± 0.21 L, respectively). One might expect

at higher PO, VTI would also increase, however, we observed increased f_B (25.2 ± 5.2 breaths/min and 22.0 ± 4.7 breaths/min, respectively) with reduced VTI, although, when taken together, resulted in higher \dot{V}_E , in conjunction with the higher $\dot{V}CO_2$ in FLK vs CONLD (Table 2). The lowered $P_{ET}O_2$ immediately post-apnea (Table 4; Figure 7A) suggests that the observed pulmonary alveoli to capillary diffusion continued during the 5 s facilitating O_2 transport. Moreover, the increased $\Delta[HHb]$ and $\Delta[HHb]/\dot{V}O_2$ in FLK-BH compared to FLK, which reflected a greater reliance on muscle deoxygenation, at the active muscle was responsible for this result (24, 25).

In elite swimmers, similar maintenance of $\dot{V}O_2$ has been demonstrated during 4 min of submaximal-intensity swimming with apnea induced by regulated breathing conditions of every two arm strokes up to a maximum of five-arm strokes, notwithstanding the reduced \dot{V}_E in the latter condition (26). The consequences of the imposed regulated breathing and comparable intensity in the current study were apparently resolved by the greater alveolar to pulmonary capillary O_2 diffusion. The muscle deoxygenation response was not recorded in this earlier work Dicker, Lofthus (26). However, a similar increase in $\Delta[HHb]$ was seen by Billaut and Buchheit (27) during 10 repeated 10 s maximal sprints followed by 30 s rest, under hypoxic conditions (13% $F_I O_2$) compared to normoxia. Conversely, others have studied the effects of repeated 3 s loadless cycling recovery periods, as opposed to the 5 s of high PO as in the present study, and observed an improved microvascular O_2 delivery reflected by decreased $\Delta[HHb]/\dot{V}O_2$ (28). It is suggested that the contrasting results of the present study were attributed to the apneic O_2 conservation effect (Kume et al., 2013), as reflected by the decreased FLK-BH $\Delta[Hb_{tot}]$ compared to FLK. This result is similar to the CONLD-BH compared to CONLD, of the present study and others, reflecting a similar redistribution of blood flow away from working muscles during apnea (1, 19). Furthermore, it is suggested that the unchanged mean $\dot{V}CO_2$ and $[La^-]$ during FLK-BH compared to FLK despite the apneic periods was a consequence of the continued buffering and pulmonary capillary to alveolar diffusion and buffering during this 5 s BH (Table 4). Conversely, Lim, Kim (1), under a similar apnea protocol (5 s), but with free breathing (25 s), as opposed to regulated breathing of the present study, showed lower mean $\dot{V}CO_2$ in FLK-BH compared to FLK and higher

lactates associated with the higher mean PO ($\Delta 50\%$ vs $\Delta 20\%$) suggesting a relative decrease in ventilatory buffering at this much higher relative PO.

This was the first study, to our knowledge, that regulated breathing during supra-threshold PO exercise on a cycle ergometer following periods of apnea, to a free breathing protocol at the same PO similar to the lower extremity work associated with swimming in the prone position. Consequentially, mean $\dot{V}O_2$ was maintained in these apneic conditions (CONLD-BH and FLK-BH) compared to their free breathing counterparts (CONLD and FLK, respectively) by greater muscle deoxygenation, despite decreased intramuscular blood perfusion. The regulated f_B and \dot{V}_E during the 25 s breathing periods of the present study eliminated the transient increases in \dot{V}_E that previously corresponded with greater post-apneic $\dot{V}O_2$ and $\dot{V}CO_2$ (1). $\dot{V}O_2$ was sustained in CONLD-BH compared to CONLD, and FLK-BH compared to FLK as a function of the continued pulmonary diffusion during apnea, as is reflected by the immediate increases in $P_{ET}O_2$ post-apnea (Table 4). At the level of the muscle, the preserved arterial O_2 content coupled with the increased muscle deoxygenation facilitated the unchanged $\dot{V}O_2$. Although these protocols were designed to replicate the breathing opportunities afforded during regulated to free breathing conditions of swimming, the cardiorespiratory responses to facial immersion, supine exercise, and the combined arm and leg muscle action specific to swimming (29-31) were not studied, due to the inherent difficulties of calculating gas exchange while submerged and the unavailability of a recumbent ergometer that would interface successfully with our data collection equipment. Additional research is needed to clarify the role of these specific swimming characteristics in these physiological outcomes. Further, our results may not reflect the responses of well-trained competitive swimmer, and as such, future studies should compare the physiological resolution of expert swimmers, compared to non-swimmers, to these breathing restrictions. Finally, only healthy participants were tested, therefore, no comparisons to diseased populations should be made.

Moreover, only male participants were included in this study to best match participant characteristics to the previous study (1). Therefore, these observations may not apply to females because of sex differences in body composition, fluctuations in reproductive

hormones (estrogen and progesterone) (32) and lactate production during exercise (33). Also, the sample size ($n=10$) of the present study adequately powered the statistical analyses used, however, a larger sample size might be required to generalize these results.

2.5 « Conclusions »

The initial and necessary reduction of PO ($\Delta 50\%$ to $\Delta 20\%$) imposed by the regulated breathing condition demonstrated the severe cardiorespiratory consequences of this regulated breathing protocol compared to the free breathing paradigm instituted previously by our lab. However, under this reduced PO, mean $\dot{V}O_2$ was maintained after the implementation of 5 s apneic periods and 25 s regulated post-BH breathing during supra-threshold exercise. The mechanism for this sustained $\dot{V}O_2$ under the apnea condition, with its reduced breathing opportunities, was expounded through an increase in muscle deoxygenation ($\Delta[\text{HHb}]$) relative to $\dot{V}O_2$ within the constraints of the O_2 conservation or deep diving response (decreased $\Delta[\text{Hb}_{\text{tot}}]$). This contrasted the systematic increases in \dot{V}_E and unchanged $\Delta[\text{HHb}]/\dot{V}O_2$ observed during free as opposed to regulated breathing conditions, under an otherwise identical apneic protocol compared to the present study. From a practical perspective, swimmers competing in the predominantly aerobic front crawl events (400 m, 800 m, and 1500 m) would be advised to increase their minute ventilation by increasing breathing frequency to twice per arm cycle as often as is comfortable, and/or increase tidal volumes, or suffer negative performance consequences.

2.6 « Future Directions »

These results identify O_2 offloading from Hb as the mechanism that maintains $\dot{V}O_2$ when breathing opportunities are limited. An apnea-driven redistribution of blood flow may accompany this resolution. However, further research is required to elucidate the contextual requirements of these mechanisms. For example, it is unknown if a similar protocol would elicit the same response when performed underwater. Also, it is unclear if periodic PPO sprints abolish the hemodynamic diving response. Future studies should investigate a similar BHing and regulated breathing protocol with a more accurate and reliable measurement of blood flow, such as positron emission topography (PET). Lastly,

female participants should be included in future studies to evaluate sex differences in these responses.

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Appendix

Ethics Approval Notice



Date: 5 January 2022

To: Glen Belfry

Project ID: 107170

Study Title: The Effects of Periodic Breath-Holding During Intermittent Exercise on Energy System Contribution, Lactate Threshold, and Regional Blood Flow Distribution.

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

Date Approval Issued: 05/Jan/2022

REB Approval Expiry Date: 08/Jan/2023

Dear Glen Belfry,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

The Office of Human Research Ethics

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

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