Study Report 2

An investigation of the ergogenic and physiological effects of ingesting a high concentration oxygen supplement on subsequent exercise performance in running.

Neil Fleming, Ph.D. (Principal Investigator) Department of Kinesiology, Recreation and Sport, Indiana State University

INTRODUCTION

The study of oxygen supplementation and exercise performance dates back to the 1940's, 50's, and 60's when high altitude climbers fought - and in many cases died - to plant their nation's flag on the highest peaks in the world. Since survival above 8000m is considered impossible, climbers used bottled oxygen to supplement their breathing in order keep them alive in the extreme environment known as "the death zone". Many studies from around this period documented the physiological effects of inspiring higher concentrations of oxygen during strenuous exercise. Breathing supplemental oxygen during exercise has been shown to increase arterial oxygen saturation [\(1\)](#page-20-0), decrease pulmonary ventilation [\(2\)](#page-20-1), lower submaximal heart rate and blood lactate values [\(3\)](#page-20-2), and increase maximal oxygen consumption or $VO₂$ max [\(4\)](#page-20-3). However, the results of breathing supplemental oxygen before or after exercise are less impressive, with several studies showing little or no benefit on performance or recovery [\(5\)](#page-20-4). Therefore, in order to benefit from supplemental oxygen, the gas must be inspired during the exercise, which is difficult if not impossible during competitive sport [\(6\)](#page-20-5).

More recently, commercially available drinks which advertise high concentrations of dissolved $O₂$ have become popular. Despite anecdotal reports from athletes and coaches, very few controlled studies have been conducted and therefore the ergogenic and physiological effects of these drinks remains questionable [\(7\)](#page-20-6). Indeed, of the limited studies which have examined the effects of oxygenated water, several have reported no effect on exercise performance [\(6,](#page-20-5) [8-11\)](#page-20-7). The majority of these studies have used $VO₂$ either at sub-maximal or maximal exercise intensity as a measure of aerobic performance [\(8,](#page-20-7) [9\)](#page-20-8), all of which showed no effect. However, it is not immediately clear why these researchers would expect to see an increase in $VO₂$ following ingestion of supplemental oxygen. $VO₂$ is calculated using the Haldane Transformation which assumes that $O₂$ consumption is equal to the difference between inspired O_2 and expired O_2 . However, ingested O_2 is not accounted for in this equation and thus any additional O_2 which reaches the circulation would not be detected using regular pulmonary gas measurement. The primary criticism put forward by skeptics of oxygenated drinks is that ingested $O₂$ - instead of inspired - is not readily diffused across the gastro-intestinal tract and into the bloodstream [\(6,](#page-20-5) [12\)](#page-20-9) and would therefore have no effect at a systemic level. However, two studies have demonstrated that highconcentration oxygen solutions are capable of diffusing $O₂$ into the bloodstream, albeit into the hepatic portal vein in rabbits [\(13\)](#page-20-10) and kittens [\(14\)](#page-20-11). Neither study assessed whether this gas diffusion altered the systemic or peripheral arterial saturation, and so extrapolating a potential ergogenic effect at a muscular level is pre-mature. One human performance study however, has reported increases in peripheral $O₂$ saturation during exercise, as a result of ingestion of oxygenated water [\(15\)](#page-20-12). The authors also reported that this increase in $SpO₂$ was more pronounced in highly trained subjects and resulted in a significant improvement in cycling performance, as measured by time to failure in

an endurance task. In another study of oxygenated water, 25 runners were tested in double-blind placebo controlled fashion using 5km time-trials. The authors reported a non-significant 15 second average improvement following oxygenated water supplementation. Once again, when the performance data from the higher trained subjects were analyzed separately, the results were statistically significant [\(16\)](#page-21-0). Both these studies appear in agreement that oxygenated water supplementation may improve performance to a greater extent in highly trained athletes. Two other published studies have observed an interesting effect of oxygenated water, related to lactate clearance kinetics [\(8,](#page-20-7) [9\)](#page-20-8). Despite both studies reporting no significant improvement in performance, the authors did report lower maximal lactate concentration and enhanced lactate clearance post-exercise. In both studies, this finding was statistically significant and it is curious that neither author discussed the potential implications of such a finding on post-exercise recovery. In addition, neither study tracked lactate clearance kinetics for longer than 6 minutes, so the full effect was never established.

Overall, the findings from studies which have examined the ergogenic effects of oxygenated water appear ambiguous. Many studies have reported no effect on either performance or $VO₂$ kinetics [\(6,](#page-20-5) [8,](#page-20-7) [10,](#page-20-13) [11\)](#page-20-14). Other studies, which recruited higher trained athletes, report improvements in performance $(7, 15, 16)$ $(7, 15, 16)$ $(7, 15, 16)$ $(7, 15, 16)$ $(7, 15, 16)$ and $O₂$ saturation (15) . Furthermore, authors have reported alterations in post-exercise lactate clearance; an important marker for recovery (8, 9). The novel aspect of the proposed study is to build on this previous work, by more closely examining the physiological effects of a high concentration oxygen supplement in a highly trained cohort. Previous studies have used peripheral pulse oximetry [\(7,](#page-20-6) [11,](#page-20-14) [15\)](#page-20-12) to examine oxygen saturation however the accuracy and specificity of this approach is limited and may explain the previous conflicting results. The proposed study will use the novel approach of examining tissue oxygenation at a muscular level using near-infrared spectroscopy (NIRS). This approach has recently been shown to be sensitive to hyperoxic gas exposure [\(17\)](#page-21-1) and is a more appropriate measure for human performance. In addition to assessing muscle oxygen saturation, the study proposes to more accurately examine the effects of oxygen supplementation on post-exercise lactate clearance. Lactate clearance is one of the most crucial measures of recovery from exercise. Any improvement in lactate clearance would enhance post-exercise recovery in athletes.

STUDY 2 UPDATE

The initial battery of tests which were performed on a cohort of 15 male cyclists proved inconclusive for drink efficacy in relation to endurance performance and post-exercise recovery. However, the high concentration oxygen supplement (OS) did show trends towards increased O_2 saturation at rest, in both the muscle tissue and peripheral capillaries, as measured via NIRS and pulse-oximetry, respectively. As such, the hypothesis that high concentration O_2 supplementation enhances the delivery of O_2 to the systemic tissues cannot be rejected and further investigation is warranted. There may be several reasons why the previous protocol did not result in detection of statistical differences in both performance and recovery capacity. Firstly, the dosage may not have been sufficient to result in a physiologically relevant increase in $O₂$ which might elicit improved performance and/or enhanced recovery. Secondly, the intrasubject variability for endurance protocols may have been too large to yield detectable differences in performance. Thirdly, the timing of dosage in the previous protocol may have limited the efficacy of the drink.

The amendments proposed for the second battery of tests will address these three issues in an effort to improve the resolution for detecting improvements in performance while still investigating the changes in systemic $O₂$ saturation. The dosage of OS will be increased from 1000mL of 3% solution, to 90mL of 100% solution. This will result in a 3 fold increase in OS dosage. Secondly, the protocol will utilize a standardized 5000m time trial as opposed to a time to failure protocol which was used in the previous battery of tests. The rationale for changing exercise protocol is that standartised time trials have a lower inter-subject variability (1-3%) compared to time-to-failure trials (3-5%) in running cohorts. A third modification of the current protocol is to provide the subjects with fluid at the mid-way point during exercise. This may improve enhance $O₂$ delivery during the exercise test, which was not possible in the previous protocol. The previous endurance trials were in some cases in excess of 20mins, time in which any physiological effects of the OS drink may have been worn out prior to the final stages of the test.

AIMS AND HYPOTHESIS

Specific aim 1: To determine if ingestion of a high concentration oxygen supplement improves maximal exercise performance. The results of previous studies have been ambiguous with regards to the performance effects of ingesting oxygenated drinks, with several authors arguing that the concentrations of available $O₂$ in solution were simply not high enough to have any effect at the muscle tissue level [\(6,](#page-20-5) [10\)](#page-20-13). However, we hypothesized that a higher concentration oxygen supplement would facilitate a significant improvement in maximal exercise performance, as measured by a decrease in time to completion (TTC) during a maximal effort 5000m time-trial performed on a treadmill.

Specific aim 2: To determine if ingestion of a high concentration oxygen supplement increases tissue O2 saturation during rest and/or maximal exercise. The proposed mechanism by which oxygenated drinks function is the diffusion of $O₂$ across the gastrointestinal tract and into the bloodstream [\(7\)](#page-20-6). While there is conflicting evidence as to the efficacy of this mechanism, measured via blood gas and/or pulse oximetry, no data currently exists at a muscle tissue level. The current study will examine muscle oxygenation via near-infrared spectroscopy (NIRS) which has recently grown in popularity as a non-invasive determinant of muscle tissue oxygenation [\(18\)](#page-21-2). We hypothesized that ingestion of a high concentration oxygen supplement would increase the oxygen saturation at a muscle tissue level during rest and/or exercise.

Specific aim 3: To determine if ingestion of a high concentration oxygen supplement improves post-exercise recovery via enhanced lactate clearance kinetics. Several studies have reported that oxygenated drinks significantly lowered blood lactate concentrations during - and following - maximal exercise (8, 9). Despite these findings, no studies have examined the lactate clearance kinetics over a sufficient time course to establish the effects on recovery. The current study will collect capillary blood samples both during and following exercise to determine any effects on lactate production and clearance. We hypothesized that ingestion of a high concentration oxygen supplement would result in lower maximal blood lactate concentrations and increase lactate clearance kinetics post-exercise, thus aiding recovery from exercise.

METHODS

Study Design

The study design for the second battery of tests was to conduct a set of double-blind, placebo controlled trials in a counterbalance cross-over design to assess the effects of ingesting a high concentration oxygen supplement on (a) performance, (b) tissue $O₂$ saturation, (c) blood lactate kinetics and (d) resting blood pressure. Since the cohort was performing identical trials following both OS and placebo ingestion, each subject acted as their own control.

Subjects

A cohort of 26 male collegiate level runners were recruited for the current study. All subjects completed a detailed health questionnaire and pre-trial screening to rule out contraindications for maximal exercise, prior to beginning the study. The anthropometric and physiological characteristics of the group are presented in Table 1.

Table 1: Anthropometric and physiological characteristics of the subject group.

Exercise Protocol

Each subject completed 3 individual trials (1 x maximal incremental trials; 2 x 5000m time trials), on a high-speed motorised treadmill (Woodway Forefront). All subjects were instructed not to eat or consume caffeine in the 60mins prior to testing, in order to ensure appropriate gastric emptying prior to ingestion of solutions and to reduce the effect that caffeine could have on exercise performance and cardiovascular function. In addition, repeat tests were performed at the same time of the day in order to minimize the effect of circadian variability on performance. All running tests were performed on the same treadmill with runners wearing a safety harness in order to eliminate the risk of falling. The Woodway Forefront treadmill is a mechanically loaded, high velocity treadmill which is accurate to ±0.1km.h-1 of velocity (see Figure 1).

Prior to initiating the pre-trial rest period, the NIRS sensor was applied to the *Rectus Femoris* and a pulse-oximeter probe placed on the middle finger of the right hand. The

recording site on the *Rectus Femoris* was shaved and cleaned with isopropyl alcohol prior to application of the NIRS sensor. In addition, the recording site was marked with permanent marker in order to ensure correct sensor placement on repeat visits. The subjects were then instructed to sit quietly for 30 mins and keep their right leg and hand as still as possible, in order to minimize motion artifact.

Figure 1: A runner exercising on the Woodway treadmill during the current study.

Trials were randomized for drink order; however the incremental trial was performed first, in order to attain physiological characteristics of each participant prior to initiating drink trials. During the drink trials, participants ingested a series of 3 x 15mL volumes of either high-concentration oxygen supplement (OS), or taste matched placebo (PL), 30 min, 15min and 5min prior to performing a 5000m time trial. Subjects were instructed to drink this 15mL volume in a mouthful but to rinse the solution around in their mouths prior to swallowing in order to enhance potential sublingual $O₂$ absorption. Participants rested in a seated position during which time tissue oxygenation, pulse-oximetry and cardiovascular function (heart rate and blood pressure) were be monitored. 15 mins into the rest period, an additional 15mL of drink will be ingested. Upon completion of the rest period participants consumed an additional 15mL of fluid (OS or PL) 5mins prior to performing the 5000m time trial.

The composition of 15mL of OS was as follows:

 ASO^\circledast solution: $\qquad \quad \mathsf{15mL} \qquad \qquad \qquad \mathsf{(100\%)}$

ASO $^{\circledast}$ solution (Activate Stabilized Oxygen) is a registered dietary oxygen supplement for human consumption. 16mL of this solution was added to 484mL of distilled water.

The ingredients to ASO $^\circledast$ are the following:

The taste-matched placebo comprised of 0.6mg of NaCl added to 15mL of distilled water. Taste testing was carried out prior to the initiation of the study. 30 students and university faculty were asked to taste both solutions and decide which one they thought was the oxygenated solution. 14 (47.5%) chose the placebo, while 16 (52.5%) correctly chose the OS solution. The majority of individuals responded that they could detect a noticeable difference between the two drinks, however they were unable to definitively separate the two.

Figure 2: Diagram of the protocol timeline. 5000m time-trials were performed in a randomized order (placebo vs. OS).

Upon completion of the 30 min rest period, subjects began a 5 min warm-up on the Woodway treadmill at a self-selected pace. Subjects were then fitted with the pulseoximeter on the middle finger of the right hand. They then performed a standardized time trial over a fixed distance of 5000m on the treadmill. Information on elapsed time and distance covered were provided continuously throughout the test via the treadmill monitor. In addition, their estimated finishing time was calculated after 1000m and feedback was provided, to allow the subjects adjust their pace. The 5000m distance was selected because the duration (15 to 18 mins) is sufficient to elicit near maximal exercise intensity and results in significant oxygen depletion. In addition, previous studies have shown that test-restest reliability for maximal time-trials is higher than submaximal endurance trials of a similar duration. Blood samples were collected from the middle finger on the left hand, in order to measure blood lactate concentration at the start, mid-point and finish of the time-trial. In addition, subject's were provided an additional 15mL volume of either OS or placebo to drink at the mid-point of the time-trial (2500m completed).

Three minutes after completion of the test, participants began a 30min passive recovery in a seated position. Subjects drank an additional 15mL volume of either OS or placebo at minutes 0 and 15 of the recovery. Throughout the recovery protocol, muscle and peripheral oxygen saturation were measured. In addition, capillary blood samples were collected every three minutes, in order to measure blood lactate concentration. This data was subsequently used to quantify post-exercise lactate clearance kinetics, a standard assessment for recovery.

Respiratory Exchange Data

Gas exchange variables were recorded using a Parvomedics TrueOne 2400 computerized metabolic cart during the maximal incremental test. This system has been shown to be an accurate device for the measurement of inspiratory and expiratory gas exchange variables [\(19\)](#page-21-3). The test-retest reliability of the TrueOne 2400 has been reported to be equivalent to Douglas bag measurements (CV $4.7 - 5.3\%$ vs. $5.3 - 6.0\%$ for TrueOne2400 and Douglas bags, respectively) which are widely regarded as the gold standard gas exchange measure [\(20\)](#page-21-4). The TrueOne 2400 metabolic cart consists of a Hans Rudolf 3813 (Kansas City, MO) pneomotachometer that measures ventilation and a gas mixing chamber system which uses a paramagnetic $O₂$ analyzer (range 0-25%) and an infrared, single beam $CO₂$ analyser (range 0-10%). Prior to each test, a calibration was performed to ensure accurate measurements from the pneumotachometer and gas analyzers. The gas analyzer calibration consists of a room air auto-calibration routine and a two-point gas calibration with a certified gas mix of 16.00% O_2 and 4.00% CO_2 [\(20\)](#page-21-4). The pneumotachometer was calibrated using a 3.00 L Hans Rudolf 5530 syringe. The calibration procedure involves an initial 5 strokes to flush the mixing chamber followed by 5 calibration strokes at varying rates within a range from 30-350L.min⁻¹. Calibrations of the gas analyzer and pneumotachometer were repeated until <0.05% difference in O_2 and CO_2 and <1.0% difference in volume were recorded.

Blood Lactate Data

Capillary blood samples were collected at the start, midway and finish of the exercise tests from the middle finger of the left hand using aseptic techniques, in order to measure blood lactate concentrations during exercise. Additional blood samples were collected every 3 mins post-exercise for a total of 30mins to assess post-exercise recovery. A 7µL sample was injected into the Analox GM7 metabolic analyzer, which measures changes in ionic gradient across a Teflon semipermeable membrane. A new Teflon membrane was installed in the Analox analyzer prior to the initiation of data collection. Test-retest analysis of the membrane was carried out via 10 repeat measures of 8mMol.L⁻¹ lactate standard. The CV for this protocol was 0.6%, which falls within the acceptable range of 0 - 1%, set by the manufacturer. The lactate analyser was calibrated prior to each exercise test, using 8mmol.L⁻¹ lactate standard. Calibration was only accepted when repeat samples measured between 7.9 and 8.1 mmol.L⁻¹.

Pulse-Oximetry

Peripheral $O₂$ saturation was measured throughout the study using an MD300M handheld pulse-oximeter. This monitor measures S_PO_2 and pulse rate at a frequency of 1Hz and stores the data for subsequent download and analysis. Data were recorded throughout all trials from the middle finger of the right hand.

Near Infra-red Spectroscopy

Muscle tissue oxygen saturation was measured continuously from the right *Rectus Femoris* using a portable NIRS monitor (Portamon MkII, Artinis; see Figure 4). The right *Rectus Femoris* was chosen over the *Vastii* muscles due to the observation of more stable data with less motion artifact during pilot testing in running. Since the midpoint of the *Rectus Femoris* is located more proximal to the hip joint than either of the *Vastii* muscles, the sensor movement was lower with this muscle. The NIRS monitor transmitted a near-infrared beam from the surface of the skin into the subcutaneous fat and muscle tissue. The rate of defraction of light back to the monitor is then used to calculate the level of oxyhemaglobin and oxymyoglobin within the underlying tissue. The final measure was reported as a combined tissue saturation index (%TSI). A 6 minute control period prior to fluid ingestion allowed for the measurement of baseline %TSI. Changes in %TSI were then normalized to this baseline for each trial. This method of estimating tissue oxygen saturation has been validated and used for the last 15 years as a measure of muscle oxygenation [\(18\)](#page-21-2).

Statistical Analysis

For comparison of data across time between the two drink trials, a 2-factor ANOVA (drink x time) with 1 repeated measure (drink) was used. Bonferoni post-hoc tests quantified significance where identified. Statistical significance was inferred at P<0.05. For variables which were independent of time (TTC, final lactate, lactate halflife etc.), paired Student's T-tests were performed. All statistical tests were performed using (Graphpad Prism Version 5).

RESULTS

Time to completion data

The group mean (\pm SD) TTC data for the time-trial were 1096 \pm 80 and 1102 \pm 93 seconds, for OS and placebo time-trials, respectively. The group performed an average of 6 seconds faster during the OS trials which represents a 0.5% improvement in performance. However, this group difference did not attain statistical significance (P=0.45, see Figures 3 and 4).

Figure 3: Group mean \pm SD time to completion (TTC) data for the OS and Placebo time-trial tests. Note that a decrease in TTC indicates an improvement in performance.

Figure 4: Individual changes in time to failure (TTC) comparing the OS and Placebo time-trial tests. Note that a decrease in TTC indicates an improvement in performance.

Blood Lactate Data

Blood lactate concentrations during the time-trials were analyzed by comparing lactate at the mid-point and finish of the trials. In addition, power at lactate threshold was calculated using the Dmax method [\(21\)](#page-21-5). Group mean lactate concentrations were lower during the OS trials at both the mid-point and finish (see Figure 5). However, no difference was detected between drink trials. Peak lactate concentration during the exercise trials were lower comparing the OS and placebo trials, however this difference was not statistically significant (6.2 \pm 1.4 vs. 6.6 \pm 2.0 for OS and placebo, respectively; $P=0.42$).

Figure 5: Lactate kinetics during the OS and Placebo trials. Data were collected immediately prior to the start, at the mid-point (2500m) and immediately following the finish.

Post-Exercise Recovery

Recovery from intense exercise is of critical importance for long-term training adaptation and improvements in performance. In the current study, post-exercise recovery was measured by examining post-exercise lactate clearance kinetics. An improvement in recovery would be evident from increased rate of clearance of lactate from the bloodstream. Initial peak lactate concentrations were lower in the OS trial, mirroring the response observed during the time-trials. Initial post-exercise lactate concentrations were 6.5 \pm 1.5 vs. 6.8 \pm 1.7 mmol.L⁻¹ for OS and placebo trials, however this difference was not statistically significant (P=0.24). Results of the 2-Factor ANOVA revealed a significant time effect (P<0.001). Despite average lactate concentration in the OS being lower at every time-point, this effect failed to attain statistical significance (P<0.168). Post-exercise recovery was also assessed by comparing the time taken to reduce the

peak lactate concentration by 50% (also known as lactate half-life). This was performed by fitting a $4th$ order polynomial over the 10 blood lactate concentrations measured following completion of the time trial and subsequently computing lactate half-life using customized algorithm written in Matlab (V7.14 R2012a, Mathworks, MA, USA). The lactate half-life was substantially lower in the OS trials, indicating higher rates of lactate clearance (1127 \pm 272 vs. 1223 \pm 334 secs for OS and placebo trials). This difference in lactate clearance was statistically significant (P<0.05).

Figure 6: Lactate clearance kinetics during the post-exercise recovery.

Heart Rate Data

Heart rate data during exercise and recovery are presented in Figure 7. No significant differences between OS and placebo were observed at any time-point.

Figure 7: Heart rate data during exercise and recovery protocols for OS and placebo.

Resting Blood Pressure

Resting blood pressure data are presented in Table 2 and Figures 8 and 9. No significant condition or time effects were observed for any of the resting blood pressure data.

Table 2: Group mean (SD) blood pressure data during pre-trial rest period. Note that 15mL volumes of either OS or Placebo were ingested at 6 and 18 mins, respectively.

Figure 8: Group mean (SD) systolic blood pressure data during pre-trial rest period. Note that 15mL volumes of either OS or Placebo were ingested at 6 and 18 mins, respectively.

Figure 9: Group mean (SD) diastolic blood pressure data during pre-trial rest period. Note that 15mL volumes of either OS or Placebo were ingested at 6 and 18 mins, respectively.

Oxygen Saturation

Oxygen saturation was measured during rest and exercise at both a peripheral capillary level using pulse-oximetry and at a muscle tissue level using near-infrared spectroscopy (NIRS). During the rest period, tissue $O₂$ saturation gradually increased however peripheral $O₂$ saturation remained stable throughout (see Figures 10 and 11). This time effect was statistically significant in both OS and placebo trials (P<0.001) and is most likely due to reduced cardiovascular stress and venous pooling associated with sitting in a chair for 30 mins. During exercise, there was a significant reduction in tissue $O₂$ saturation across time (P<0.001, see Figure 12), however no differences between drink trials were observed. Tissue $O₂$ saturation did appear lower during exercise however the differences to placebo were not significant (P=0.307).

Figure 10: Group mean \pm SD muscle tissue saturation data during rest prior to OS and placebo trials. Data were normalized to 6 minutes baseline data collected prior to ingestion of the first 15mL volume. The horizontal axis indicates the time following ingestion of either 15mL of OS or placebo. Note that an additional 15mL volume of OS or placebo was ingested at minute 12.

Figure 11: Group mean \pm SD peripheral saturation data during rest prior to OS and placebo trials. The horizontal axis indicates the time following ingestion of either 15 mL of OS or placebo. Note that an additional 15mL volume of OS or placebo was ingested at minute 12.

Figure 11: Group mean \pm SD muscle tissue saturation data during OS and placebo exercise trials. Data were normalized to 6 minutes baseline data collected prior to ingestion of any fluid. The horizontal axis indicates the time during the test. Data were recorded using NIRS monitor.

CONCLUSIONS

The main finding from the current battery of tests is that ingestion of OS significantly improved post-exercise recovery from high intensity aerobic exercise, via enhanced lactate clearance. However, this enhanced metabolism of lactate did not yield an improvement in performance during exercise. The initial battery of tests which were conducted on a group of cyclists using a lower concentration OS did not result in improved performance during maximal incremental exercise or increase TTF during submaximal endurance exercise in cycling. It was subsequently hypothesized that the OS was not of a sufficiently high concentration to facilitate sufficient diffusion of $O₂$ into the systemic circulation, necessary for the working muscles. As a result of the initial study, the protocol for the current study was modified to provide a 3-fold higher dosage of OS prior to exercise. In addition, OS was provided during exercise in order to improve the efficacy of dosage and a 5000m time trial was used as it more closely replicates competitive racing. While these modifications did not result in a detectable improvement in performance, drinking OS did result in a statistically significant improvement in postexercise, which is an important finding.

Training is essentially the cyclic process of physiological stimulus and recovery. Repetition of this process over a period time elicits a training adaption. Enhancing postexercise recovery from training is of significant benefit to competitive athletes, as it is likely to increase the rate of training adaptation in the long term. Additionally, many athletic events over short and middle distances (even up to 5000m) require the multiple races over the course of a competition. The ability to clear lactate more efficiently and hence recover faster in the early rounds of competition is of clear benefit to an athlete. Post-exercise recovery is also of significant importance in team based sports. For example, the sport of basketball involves continuous flow of play with players performing high intensity movements on average every 21 seconds (22). However, basketball players receive regular recovery during the game via substitution, time-outs and breaks at each quarter. Despite these periods of recovery it has been shown that players compete with average circulating blood lactate concentrations of 6.8 mmol. $L⁻¹$ throughout the game (22). Any intervention which may enhance the clearance of lactate during a player's recovery would therefore likely improve overall performance in this sport.

Previously, it had been reported that ingestion of oxygenated water can improve aerobic performance and significantly increase post-exercise lactate clearance [\(9\)](#page-20-8). While the current data observed a minor (6 second) improvement in performance, ingestion of OS did increase post-exercise lactate clearance, which is in agreement with previous research (9). However, it remains to be elucidated the physiological mechanism by which ingestion of OS enhances lactate clearance. It may be due to increased intra- and extra-cellular shuttling of lactate via MCT-4 or MCT-1 transporters, respectively (23).

Alternatively, increased transport and subsequent oxidation of pyruvate via enhanced mitochondrial activity in the muscle tissue may also explain this result. Further investigation is necessary in order to identify the mechanism for increased lactate clearance. While no differences in tissue $O₂$ saturation were observed during rest, exercising tissue O_2 saturation was consistently lower during exercise (see figure 11), however this effect was not statistically significant (P=0.307). It is not immediately clear why ingestion of OS may result in the appearance of lower tissue $O₂$ levels.

It should be noted that the aerobic fitness level of the current cohort was substantially higher than in the previous study. It has previously been suggested that higher level athletes responded better to OS than untrained individuals (15). This may go some way to explaining the improved results of the current study. A comparison of the current results and the initial results suggests that increasing the OS dosage in the current study had a positive effect. Overall, the results of the current study illustrate that ingestion of OS significantly improved post-exercise recovery from a maximal 5000m time-trial. The majority of participants improved their performance in a 5000m time-trial and showed significantly enhanced recovery post-exercise. On average, the group improved 6 seconds in the time-trial and showed significantly more rapid clearance of lactic acid, by an average of 96 seconds.

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