THE EFFECTS OF ACTIVATED STABILIZED OXYGEN ON AEROBIC ENDURANCE IN DIVISION II COLLEGIATE MALE SOCCER PLAYERS

by

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ABSTRACT

THE EFFECTS OF ACTIVATED STABILIZED OXYGEN ON AEROBIC ENDURANCE IN DIVISION II COLLEGIATE MALE SOCCER PLAYERS

Peter J. Fuller

This study was designed to compare aerobic endurance performance in Division II male soccer players under the conditions of Aquafina bottled water (placebo) and Activated Stabilized Oxygen (ASO). Participants in this study were the members of the Humboldt State University Men’s Soccer team (N=12) with the fastest two-mile run times. The study was performed using a randomized, double-blind, cross-over design. Two VO_{2max} trials were conducted. The hypothesis was that ASO would result in significantly higher %SpO_2, time to exhaustion and other aerobic performance measures. The %SpO_2 (95.58 ± 3.05 vs. 96.08 ± 2.46; p > .05), HR (187.00 ± 8.66 vs. 187.16 ± 7.73; p > .05), and VO_2 (ml/kg/min) (57.26 ± 4.93 vs. 58.15 ± 3.87; p > .05) at the highest common workload (HCWL) achieved on both trials were not different between the placebo and ASO conditions, respectively. The %SpO_2 at exhaustion (94.75 ± 3.01 vs. 95.16 ± 2.24; p > .05) and time to exhaustion (428.83 ± 62.54 vs. 451.17 ± 57.05; p > .05) were not different between conditions. The %SpO_2 at the lowest point during the max testing (94.58 ± 2.90 vs. 94.92 ± 2.06; p > .05), VO_{2max} (ml/kg/min) (59.97 ± 4.24 vs. 60.92 ± 3.51; p > .05) and maximum HR (192.08 ± 7.69 vs. 191.08 ± 7.63; p > .05) also were not significantly different between the conditions. Although no significant differences were found in any of the measurements, it should be noted that time to exhaustion was higher by 23.34 seconds (p = .072) in the ASO condition compared to the placebo. In conclusion, the consumption of ASO did not
produce any significant changes in aerobic endurance performance in Division II male soccer athletes.
ACKNOWLEDGEMENTS

My most sincere gratitude goes to the chair of my committee, Tina Manos. I want to thank you for introducing me to the marvels and frustrations of scientific research. Thank you for your positive encouragement and endless energy to push me in the right direction. I would probably be still writing if it were not for your commitment to getting me out of here. I also want to thank Kathy Munoz and Thomas “TK” Koesterer, for their insight and suggestions along the way. I want to express my sincere gratitude to those who helped in the Human Performance Lab (Pat Hyland, Susie Deimer, and Tom Herr). To my family, thanks for always being there with your love and support. Finally to Ellie, thank you for being there with me through all these times and putting up with my messes around the house.
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11. Comparison of tidal volume between the placebo group and ASO group during treadmill testing procedures.
A fundamental problem in exercise physiology is the identification of those factors primarily responsible for hampering human performance (Wilson, Welch, & Liles, 1975). Identifying the cause of fatigue in human performance (e.g., the inability to maintain a given exercise intensity) is not that simple, as it is often difficult to separate causality from concurrent appearance (Brooks, Fahey, & Baldwin, 2005). The failure of one enzyme system, cell, or muscle group is likely to affect numerous other cells, organs and tissues (Brooks et al., 2005). A number of potential causes for fatigue during exercise have been identified. They include inadequate strength or skill level, adverse environmental conditions (altitude, heat, and humidity), limits to the CNS function (arousal and motivation), dietary factors (carbohydrate, fat, and water intake), depletion of metabolites (glycogen, blood glucose, ATP and CP), accumulation of metabolites (lactic acid, H\(^+\), and Ca\(^{2+}\)), as well as the depletion of muscle O\(_2\) stores, or rather the inadequacy of the circulating O\(_2\) that needs to be delivered to the muscles (Brooks et al., 2005; Powers & Howley, 2007). Individuals who participate in strenuous exercise at sea level can fall short in the balance between muscle respiratory requirement and O\(_2\) supply. Cell respiration and a sufficient O\(_2\) supply are essential in activities lasting longer than 90 seconds to produce the required ATP for activities that require a work rate close to 100% VO\(_{2}\)max (Brooks et al., 2005).
An insufficient amount of oxygen during exercise can result in fatigue and impair muscle contractions (Brooks et al., 2005). With the lack of oxygen during exercise comes an increase in lactate concentration in the body (Hargreaves & Thompson, 2001). At one time lactate was thought to be the conspirator which caused a decrease in performance. According to Brooks et al. (2005) it is actually the accumulation of hydrogen ions (H\(^+\)) which hinders performance. When lactate dissociates it forms H\(^+\), which can cause a decrease in pH and an increase in acidity. The increase in acidity will eventually cause acidosis, which has the negative effect on muscle contraction, performance and the production of ATP (Hargreaves et al., 2001).

Researchers agree that the negative effect on performance associated with blood lactate accumulation is due to an increased amount of H\(^+\) (Brooks et al., 2005; Hargreaves and Thompson, 2001; Powers & Howley, 2007).

Oxygen’s role in the production of ATP is linked to the electron transport chain (ETC). The oxidative energy system allows pyruvate produced by glycolysis to cycle through the Krebs cycle and the ETC. In the ETC, high energy electrons are stripped off of hydrogen and accepted by oxygen (the final electron acceptor), allowing ATP to be produced (Brooks et al., 2005). Insufficient oxygen during the ETC causes H\(^+\) to accumulate and pH to decrease, which in turn will halt oxidative phosphorylation (Powers & Howley, 2007). The production of ATP will be left up to anaerobic metabolism, which is not efficient over long periods. The effects of an inadequate oxygen supply are characterized by increased levels of lactate and/or a drop in creatine phosphate (CP) levels (Brooks et al., 2005). With the lack of oxygen to the active muscles the two aforementioned fatigue-causing effects can drastically alter or stop performance within seconds or minutes, depending on the conditioning level of the athlete (Brooks et al., 2005; Janssen, 2001).
In addition to the role O\(_2\) plays in producing ATP, which lessens the reliance on anaerobic pathways, having sufficient O\(_2\) in the blood can also decrease pulmonary ventilation and reduce the work of breathing (Powers & Howley, 2007; Welch 1987; Wilson & Welch, 1975; Wilson & Welch, 1980). For those reasons, maintaining or increasing the partial pressure of oxygen (PO\(_2\)) in the blood should lead to an enhancement in performance in endurance athletes. Increasing oxygen content to the body can be achieved by breathing hyperoxic mixtures of air. Hypoxia is defined as a condition in which the inspired oxygen pressure is greater than that of air at sea-level, but not more than one atmosphere of absolute pressure (Welch, 1987).

The use of oxygen during exercise to enhance the level of performance is based on the notion that the muscle is hypoxic during exercise and additional oxygen will diminish the problem (Powers & Howley, 2007; Welch, 1987). The supplementation of oxygen increases PO\(_2\), which slows glycolysis during heavy exercise, resulting in a slower accumulation of lactate and H\(^+\) in the plasma; consequently, extending time to exhaustion (Hogan, Cox, & Welch, 1983; Powers & Howley, 2007; Welch, 1987). While researchers have shown little or no benefit in the breathing of supplemental oxygen before or after exercise (Elbel, Ormond, & Close, 1962; Robbins, Gleeson, & Zwilich 1992; Winter, Snell, & Stray-Gundersen, 1989), there is ample evidence that breathing hyperoxic mixtures of air during exercise enhances performance during laboratory trials, yet, breathing hyperoxic air is not practical in the field or during training (Jenkins, Waddell, Moreland, & Fernhall, 2001).

Welch (1987) reports that hyperoxic gas supplements increased performance in endurance events with times greater than two minutes and less than 30 minutes. Breathing oxygen-enriched gas mixtures of 60 to 100% during exercise can increase time to exhaustion by 20 to 40% in both runners and cyclists (Welch, 1987). While Welch (1987) reports that
hyperoxic conditions, in general, can only increase $\text{VO}_{2\text{max}}$ by 2 to 5% during exercise. Byrnes, Mihevec, Freedson, and Horvath (1984) reported an increase in $\text{VO}_{2\text{max}}$ as substantial as 13%. With regard to submaximal exercise, Welch, Mullin, Wilson, and Lewis (1974) reported a 30% increase in $\text{VO}_2$ and a decrease in lactic acid while breathing 80% oxygen during exercise at 90% $\text{VO}_{2\text{max}}$. Margaria, Ceretelli, Machi, and Rossi (1961) showed a 10% increase in $\text{VO}_2$ while inspiring 100% oxygen during submaximal exercise. Wilson et al. (1975) showed a significant increase in $\text{VO}_2$ while breathing a 60% oxygen mixture during bicycle ergometer rides at both 40% and 80% $\text{VO}_{2\text{max}}$.

Numerous researchers have found that the breathing of supplemental oxygen during exercise enhances performance (time to exhaustion) with an increased arterial oxygen content, decreased pulmonary ventilation, lower submaximal heart rate, and blood lactate values, and a higher $\text{VO}_{2\text{max}}$ (Bannister & Cunningham, 1954; Cunningham, 1966; Ekblom, Huot, Stein, & Thorstensson, 1975; Hughes, Clode, Edwards, Goodwin, & Jones, 1968; Dempsey 1986; Duncan, 1997; Hill, Long, & Lupton, 1924; Margaria, Edwards, & Dill, 1933; Miller, Perdue, Teague, & Ferbee, 1952; Tait, 1969; Welch, Mullin, & Welch, 1975; Welch, 1982; Wilson & Lewis 1974; Wilson, Wilson, Welch, & Liles, 1975). Nevertheless, Welch (1987) reported flaws in the earlier studies. The authors of the studies often do not report such controls as randomization of the treatments or of keeping the subjects naive as to the gas mixtures being utilized (Cunningham, 1966; Hughes et al., 1968; Miller et al., 1952; Tait, 1969). On one occasion, Miller et al. (1952) informed the subjects of the type of oxygen they were inhaling. That said, there are several well-controlled studies in which a positive effect in performance occurs in endurance activities when hyperoxic mixtures are breathed in during exercise (Welch,
1982). This triggers the following question; if O$_2$ is delivered by other means, for example, the ingestion of oxygenated water, can it increase endurance performance?

Over the past several years, super-oxygenated waters have become available on the market. Some of the makers of these oxygen-enhanced waters advertise them to contain seven to 30 times more oxygen than normal tap water. Marketers promote these products as being able to increase the availability of oxygen to the active tissues and enhance performance. Theoretically, the extra oxygen consumption could potentially have effects on performance similar to breathing hyperoxic gas mixtures, however, super oxygenated water is a relatively new product. Very few studies have been conducted, and therefore the effects remain questionable.

Cooper, Smith, and Pask (1960) conducted an early study to determine whether the introduction of oxygen in ingested water could be transported into the portal vein from the gastrointestinal tract. Oxygenated saline solution was passed via catheter into the stomach and small intestine of anaesthetized kittens. Cooper et al. (1960) found there was no significant amount of oxygen transfer from the gastro-intestinal tract to the systemic arterial blood, although oxygen absorbed from the small intestine was known to travel to the liver via the portal vein. The PO$_2$ of mesenteric venous blood was increased by 9-14% after treatment. Cooper et al. (1960) also concluded the oxygenated saline solution was used as the source of oxygen by the lumen of the intestine, but it was not actually available in the capillary circulation. This suggested the oxygen was used by the mesenteric tissue and the liver for its own metabolic needs (Cooper et al., 1960).

Forth and Adam (2001) conducted a study using oxygenated water where anaesthetized rabbits were intragastrically administered 30 ml of water containing 45, 80 or 150 mg O$_2$ L$^{-1}$ of oxygenated water (tap water contains approximately 7-15 mg O$_2$ L$^{-1}$ of dissolved oxygen). After
administration of the 80 and 150 mg O$_2$ L$^{-1}$ solutions of water, PO$_2$ in the portal vein resulted in a significant increase by 14 mm Hg. Forth and Adam (2001) also reported that upon awakening, the rabbits’ supplemental oxygen was available to support the increased metabolism in the abdominal cavity. Yet, no measures were made of systemic arterial oxygen content. Forth and Adam (2001) did substantiate that gas diffusion laws and temperature changes affected oxygen concentrations in the expected direction.

While it is not clear if PO$_2$ values in arterial circulation (which would be available to the working muscles) are increased with ingested oxygenated water, the possibility has not been completely discounted. Researchers have been interested to see if oxygenated water could supply enough oxygen to the athlete to increase their performance. Several researchers have found no statistically significant improvement in VO$_{2\text{max}}$, VO$_{2\text{submax}}$, blood lactate levels (BL), arterial blood saturation of oxygen (SaO$_2$), heart rate (HR), or blood pressure (BP) when oxygenated water was ingested (Askew, Pfeiffer, Reading, & Ensign, 2000; Hampson, Pollock, Piantadosi, 2003; Leibetseder, Straus-Blasche, Marktl & Ekmekcioglu, 2006; Mielke, 2004; Willmert, Porcari, Foster, Doberstien, & Brice, 2001; Willmert, Porcari, Foster, Doberstien, & Brice 2002; Wing, Askew, Luetkemeier, Ryu Jin, Kamimori, & Grissom 2003; Wing-Gaia, Sunbdhui, & Askew 2005). The methodologies and effect on dependent variables for each of these studies are summarized in Table 1. However, there are, two groups of proprietary researchers (Pericleous, 2000; Zaheeruddin & Hennessy, 2000) who have shown beneficial effects of oxygenated water on athletic performance (i.e., 60% VO$_{2\text{max}}$ for 40 minutes, 80% VO$_{2\text{max}}$ until exhaustion, and 200 m sprint time). Although neither of the studies had statistical significance to report. The quality and controls in the proprietary studies are dubious; hence, the literature is not summarized here. Lastly, there are two controlled research studies in which there was a significant effect on two of
Table 1

Reference Chart of Articles

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<tr>
<th>Authors</th>
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<th>Participants</th>
<th>Measurements</th>
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<tr>
<td>Duncan (1997)</td>
<td>20 male; 5 female</td>
<td>VO2max on Bicycle Ergometer</td>
<td>Subjects were moderately trained and exercise &gt; 4 days a week.</td>
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these variables, at least in a subset of the subject group (Duncan, 1997; Jenkins, Waddell, Moreland, & Fernhall, 2001).

Jenkins et al. (2001) conducted a randomized double-blind study and found that individuals who were moderately trained (VO$_2$ max $>$ 45 ml/kg/min) may benefit from the consumption of oxygenized water. The SaO$_2$ levels during acute bouts of intense exercise were increased under the condition of oxygenated water. Twenty subjects (10 women and 10 men), who exercised four or more days a week, volunteered for the study. Each participant completed two maximal tests and two endurance tests (at maximum work rate) on a cycle ergometer, two for each water condition (distilled and oxygen enhanced). Fifteen minutes before each test, the subjects consumed 500 ml of water (distilled water or oxygen enhanced water). A significantly higher SaO$_2$ level was found in the entire group of subjects at the end of the endurance test under the condition of oxygenated water vs. the placebo (91.31% vs. 87.3%; $p < .05$). When only those with VO$_2_{max}$ $>$ 47 ml/kg/min were examined, the researchers found SaO$_2$ at the end of the endurance test was higher with oxygenized water ($p < .05$) and time to fatigue during the maximal exercise test was 28 seconds greater with the oxygenized compared to the distilled water (1088 vs. 1060 sec; $p < .05$). Jenkins et al. (2001) suggest that well trained individuals could significantly benefit with oxygenized water, even with small increases in oxygen saturation levels.

In an unpublished study, Duncan (1997) evaluated fluid replacement with oxygenated water and the possible physiological and biochemical benefits during exercise. Duncan (1997) examined the effect oxygenated water would have on a 5K (3.1 mile) time using a randomized double-blind crossover design. The 25 active subjects had a history of completing at least one marathon and were currently running at least 30 miles a week. The subjects (5 female and 20
male) were given 18 bottles (400 ml) of standard bottled water or oxygen-enhanced water (50 mg O$_2$ L$^{-1}$ or 100 mg O$_2$ L$^{-1}$) in a randomized double-blind manner and instructed to drink three bottles per day for the six days preceding laboratory testing. Each participant was evaluated on three occasions (baseline, one week after baseline, and two weeks after baseline). The authors reported that 83% of the fastest times were in the trial in which oxygenated water was ingested ($\chi^2 = 9.78; p < .01$). The ingestion of the oxygenated water, although not statistically significant, was linked with a 15-sec faster overall 5K time trial compared to the control condition (water). Also, among a subset of highly fit men and women (VO$_{2\text{max}}$ > 54 ml/kg/min), oxygen-enhanced water significantly decreased 5K running time by an average of 31 seconds compared to the placebo condition (Duncan, 1997).

Jenkins et al. (2001) and Mielke (2004) were the only researchers testing under normoxic environmental conditions to measure SaO$_2$ levels during and after exercise testing. Jenkins et al. (2001) were unique in reporting higher SaO$_2$ levels in the subjects who ingested the oxygenated water compared to the regular water, while Mielke (2004) failed to find any significant differences. It could have been that the subjects in the study by Jenkins et al. (2001) desaturated and the oxygenated water improved their SaO$_2$ levels. Current research suggests that desaturation (SaO$_2$ of < 91% during exercise) occurs in approximately 50% of elite endurance athletes (Dempsey, Hanson, & Henderson, 1984; Powers, Dodd, & Woodyard, 1984; Williams, Powers, & Stuart, 1986). Dempsey et al. (1984) and Williams et al. (1986) believe the desaturation phenomenon in elite athletes is due to diffusion limitations as well as the short red blood cell travel times in the pulmonary capillary bed. Harms et al. (2000) shows that athletes who desaturate under normoxic conditions have the greatest improvements in VO$_{2\text{max}}$, while breathing hyperoxic gas. This implies the benefits of supplemental oxygen to performance are best noted in
athletes who will have the greatest drop in SaO$_2$ from rest during exercise. To date, there has not been any research published on testing the potential benefits of oxygenated water in athletes who have specifically exhibited exercise-induced hypoxemia. According to Powers (1988) SaO$_2$ levels in healthy adults do not change until subjects are at 75-80% of their VO$_{2\text{max}}$.

A common thread shared between the Duncan et al. (1997) and Jenkins et al. (2001) studies were that test subjects had high VO$_{2\text{max}}$ values. Jenkins et al. (2001) showed a significantly higher SaO$_2$ level in the entire group of subjects at the end of the endurance test under the condition of oxygenated water (91.31% vs. 87.3%; $p < .05$). When only those with VO$_{2\text{max}} > 47$ ml/kg/min were examined, the researchers concluded the SaO$_2$ levels at the end of the endurance test was higher with oxygenized water ($p < .05$) and time to fatigue during the maximal exercise test was 28 seconds greater with the oxygenized compared to the distilled water (1088 vs. 1060 sec; $p < .05$). Duncan (1997) showed a significant decrease in 5K running time by an average of 31 seconds in subset with VO$_{2\text{max}} > 54$ ml/kg/min ($p < .05$). Although Mielke (2004), Willmert et al. (2001), and Willmert et al. (2002) also had subjects with a VO$_{2\text{max}} > 45$ ml/kg/min, their subjects did not show any significant differences between conditions of oxygenated water and bottled water. One reason could have been the quality of the oxygenated water used in the studies. While Duncan et al. (1997) and Jenkins et al. (2001) did not have water samples evaluated for oxygen content, Willmert et al. (2001), Willmert et al. (2002) and Mielke (2004) had their water samples analyzed. In all three of the latter studies, the oxygenated water content was below that of the manufacturer’s claim. Willmert et al. (2002) reported the oxygenated water only had three times the amount of oxygen compared to regular tap water versus ten times the amount as the manufacturer had claimed. All researchers who examined the oxygenation level of the water found the water samples to contain less oxygen than the
manufacturers claim (Hampson et al., 2003; Leibetseder et al., 2006; Mielke, 2004; Willmert et al., 2001; Willmert et al., 2002).

While it is widely accepted that using hyperoxygen gas mixtures (60 to 100%) during endurance exercise enhances performance, the credibility of oxygenated water as an ergogenic aid is still uncertain. As we have seen, there is conflicting evidence regarding oxygenated water and its role in athletic performance. To our knowledge, no persons have examined the effect of oxygenated water in athletes with both a high $\text{VO}_2\text{max}$ and who have been shown to desaturate with exercise. Therefore, the aim of this study is to examine the effects of oxygenated water on aerobic performance measures during $\text{VO}_{2\text{max}}$ testing in Division II male intercollegiate soccer players who are aerobically fit. If the oxygenated water condition results in improved $\%\text{SpO}_2$ levels and time to exhaustion, for example, this may have important implications for the type of water an athlete will ingest during pre-training or pre-performances.

Significance/Statement of the Problem

It is not known if the oxygenated water, Activated Stabilized Oxygen (ASO), can improve endurance performance in Division II male intercollegiate soccer players. For example, can ASO improve $\%\text{SpO}_2$ levels, and time to exhaustion during $\text{VO}_{2\text{max}}$ testing? The purpose of this research is to compare $\%\text{SpO}_2$ levels, time to exhaustion, and other aerobic performance measures during $\text{VO}_{2\text{max}}$ testing between conditions of oxygenated water or bottled water in this specific group of athletes.
Substantive Hypothesis

The ingestion of oxygenated water will significantly increase %SpO₂, time to exhaustion, and other aerobic performance measures in Division II male soccer players.

Assumptions

For the current study, assumptions made, included: (1) All athletes were prepared for fitness testing. (2) All athletes performed to the best of their ability, (3) The lab equipment was fully functional, (4) Temperature was consistent throughout the trials, (5) track and weather conditions did not affect 2-mile run time scores, (6) The water and food intake of the subjects stayed consistent throughout the experimental testing, and (7) Workouts prior to testing were the same for each subject in the days preceding each of the VO₂max tests.

Practical Applications

Athletes, trainers and coaches are looking for an edge to gain on their opponents. Increasing performance during a timed event, even by a millisecond, can mean the difference between first and second place. Breathing oxygen in a laboratory setting has been shown to increase VO₂max, time to exhaustion, and SaO₂ levels, and decrease blood lactate (BL) and heart rate (HR). However, breathing hyperoxic oxygen on the field is not practical. The question remains as to if ingestion of oxygenated water will improve the aerobic performance of athletes. If the oxygenated water results in an improvement in SaO₂ and time to exhaustion, then this may have important implications for the training of and performance of endurance athletes. As we all know, the smallest increase in performance may be significant in the outcome of an event.
CHAPTER TWO

Methods

Participants

Approximately 20 collegiate Division II male soccer players from Humboldt State University (HSU) (Arcata, CA) were recruited for the study. The participants were tested during their off-season when the focus of training was on physical conditioning and individual technique. The training schedule of the athletes at the time the study was conducted and shown in Appendix A. All training sessions were supervised by the coaching staff and/or strength and conditioning coach.

Prior to any measurements or testing, all subjects read and signed informed consent forms to participate in the study (Appendix B). Information regarding age, playing position, years of playing experience at collegiate level, and medical history were collected prior to participation via questionnaire (Appendix C).

Participants were screened for inclusion and exclusion criteria. The medical history questionnaire was used to screen the athletes for cardiovascular disease risk factors and medical history. Only the athletes who were of “low cardiovascular disease risk” according to the American College of Sports Medicine (ACSM, 2010) were eligible to participate in the study. If the athlete did not know his blood cholesterol levels he was assumed to have a value within the normal range. Any potential participant who indicated that he has had or has a musculoskeletal injury or medical condition that could become worse by participation in strenuous activity was excluded from the study. Each subject who was eligible (N=20) to participate performed a 2-mile run to determine if they would qualify to continue in the experimental trials. To continue in the
study the subjects were required to have completed the 2-mile run and finished in the top 12 of the fastest times.

Experimental Design and Procedures

The study was performed using a randomized, double-blind, cross-over design. A series of three tests were conducted, including an initial 2-mile timed run (2-mile run), and two VO$_{2\text{max}}$ tests. Subjects performed all three tests on three separate occasions, in which their regular training remained constant. For the 2-mile run, all subjects reported to the Redwood Bowl at HSU and performed the 2-mile run at the same time. Four weeks following the initial field test, the 12 fastest athletes reported to HSU’s Human Performance Lab (HPL) to complete the initial VO$_{2\text{max}}$ test on a treadmill. The subjects performed the same VO$_{2\text{max}}$ test 7 to 10 days later and at the same time of day as the first VO$_{2\text{max}}$ test. Prior to VO$_{2\text{max}}$ test, the athletes ingested oxygenated water (ASO) or Aquafina® (PepsiCo. Inc., United States) purified bottled water. The subjects repeated the VO$_{2\text{max}}$ test using the opposite water condition on the second visit to the HPL.

Height/ Weight

A calibrated scale (Health-o-Meter, Illinois) was used to measure height to the nearest 0.5 cm and weight to the nearest 0.5 lb. All participants wore the same practice gear during weight and height measures. This included a short-sleeved t-shirt, shorts and bare feet. Their standing height was measured according to procedures slightly modified from those used in the National Health and Nutrition Examination Survey (NHANES) using a stadiometer (Health-o-Meter, Illinois). Procedures for collection of standing height included standing on the floor with the participant’s back to the backboard. The participants stood so that the posterior of the heels, buttocks, shoulder blades and head were touching the wall. Participants were positioned by the
principal investigator into the correct Frankfort plane. A deep breath was taken and the height of
the athlete measured. Immediately following the height measurements, the subject’s weight was
taken.

2-mile run

The 2-mile run test was used to identify subjects who were likely to have the highest
$\text{VO}_2\text{max}$ values. The time trial was performed in the HSU Redwood Bowl on a 440 yard running
track. It is widely accepted that a maximal effort 2-mile run is a valid indication of a person’s
aerobic fitness level, determined by $\text{VO}_2\text{max}$ (Mello, Murphy, & Vogel, 1988). Theoretically, an
athlete who runs this distance in a shorter time is able to use oxygen to a greater extent to
generate ATP and therefore requires less anaerobic ATP production.

Athletes completing a 2-mile run were expected to run this distance at a pace that is in
excess of 75% of their $\text{VO}_2\text{max}$. At this relative oxygen consumption, athletes receiving
supplemental hyperoxic oxygen gas had an improvement in time to exhaustion, $\text{SaO}_2$ levels and
$\text{VO}_2\text{max}$ in studies by Margaria et al. (1961), Welch et al. (1974), Wilson et al. (1975), Byrnes et
al. (1984), and Welch (1987). Powers and Howley (2007) suggest that athletes may experience
desaturation at this level.

On the day of the 2-mile run, subjects reported to the HSU Redwood Bowl. The subjects
were told to show up at their regular practice time of 1:45 pm on Wednesday and to go through
their routine warm-up and stretches lead by their team captain. The assistant coach instructed the
players to listen for their time as they complete each lap. A demonstration was done so the
subjects could hear and see how the run was to be performed. The participants started as soon as
the assistant coach yelled “Go.” At that point, the assistant coach pushed the button on the
stopwatch with his index finger (Baechle, et al., 2008). The subjects were notified of the elapsed
time as the assistant coach yelled out the time each time the subjects completed a lap. The
subjects reported the final time to the principal investigator (Peter Fuller, PI) upon completing
their eighth lap. The PI then recorded the athlete’s time. Times were recorded to the nearest
second. Once the participants completed the test, they were encouraged to cool down and stretch
on their own accord. The 12 fastest runners on that day were invited to participate in the
experimental trials with oxygenated water and placebo.

Pretest Conditions

Diets were not analyzed, but the athletes were asked to record their food and beverage
intake for the 36 hours prior to the first VO\textsubscript{2max} test. The athletes were asked to replicate food and
beverage quantity and time eaten in the 36 hours prior to the second VO\textsubscript{2max} test. In addition, the
athletes were asked not to eat 1.5 hours prior to any of the tests. The participants were also
reminded to eat ample amounts of carbohydrates and to consume plenty of water 36 hours prior
to testing. The participants were also given a dietary form (Appendix D) to record their diet 36
hours prior to the first VO\textsubscript{2max} test. The participants handed in their dietary form the day of the
first VO\textsubscript{2max} test to the principal investigator so that copies could be made. The PI handed back
the diet forms after copies were made and 48 hours before the second VO\textsubscript{2max} test so the
participants would have a copy for a reminder.

Maximal oxygen uptake test

According to Kraemer (2008), VO\textsubscript{2max} is the maximum volume of oxygen that can be
utilized in one minute during maximal exercise. It is measured as milliliters of oxygen used in
one minute per kilogram of body weight. This measurement is generally considered the best
indicator of an athlete's cardiovascular fitness and aerobic endurance. The criteria for achieving
VO\textsubscript{2max} is characterized by a plateau in oxygen uptake with a further increase in work rate, and/or
a post-exercise blood lactate level of > 8mmolL⁻¹ (Powers & Howley, 2007). To avoid the invasiveness of blood sampling for lactate, McKinnen and Daniels (1976) and Williams, Powers, and Stuart (1986) propose yet another valid measure of VO₂max. They proposed that a valid VO₂max can be evaluated if any two of the following criteria are met: 1) respiratory exchange ratio (RER) value is equal to or exceeds 1.15, the heart rate (HR) during the last exercise stage is ± 10 beats per minute of the subjects age-predicted HR, or 2) a plateau in VO₂ occurs with an increase in work rate (Powers & Howley, 2007). The latter set of criteria will be used to determine that a valid VO₂max value is attained for each athlete.

The VO₂max test in this study was performed on a treadmill using the Astrand and Rodahl protocol (Astrand & Rodahl, 1986). Fifteen minutes before the test was to take place, the participants drank 500 ml of the assigned water treatment. Heart rate (HR) and %SpO₂ were monitored (Nellcor Oximax N-560, Canada) and recorded every two seconds throughout the testing. Prior to beginning the Astrand and Rodahl Protocol (Astrand & Rodahl, 1986), the subjects were connected to all the instrumentation on the treadmill to warm-up at a pace of 7 mph for five minutes. After the warm-up, the subjects started the testing at a pace of 8 mph with a 2.5% gradient for two minutes. Thereafter, gradient was systematically increased by 2.5% every two minutes until volitional exhaustion. The subject’s time to exhaustion, %SpO₂, VO₂max, HR, VO₂, rate of elimination of carbon dioxide (VCO₂), expired ventilation (Vₑ), and respiratory exchange ratio (RER) were recorded. Following volitional exhaustion the subjects cooled down at a walking pace for five minutes at 0% gradient, during which time blood pressure and HR was monitored to insure these values stabilized before the athlete stopped the test (ACSM, 2010). One week later, subjects returned to the Human Performance Lab at the same time as the prior week. They repeated the same protocol with the opposite water condition.
Oxygen saturation of arterial hemoglobin

Oxygen saturation of arterial hemoglobin was measured with the Nellcor Oximax N-560 Pulse Oximeter (Canada) every 2 seconds of exercise. The Nellcor Oximax N-560 Pulse Oximeter (Canada) has been tested for accuracy during laboratory testing on a treadmill (NONIN® Medical, Inc). Preparation for the forehead sensor included cleaning the forehead (above the right eyebrow) with Medline Alcohol Swabs thick 2-ply pad (Canada) to insure electrical continuity with the Oximax MAX-FAST® adhesive forehead reflectance sensor. The sensor was then placed above the subject’s right eyebrow and secured with the headband. The Nellcor Oximax N-560 Pulse Oximeter (Canada) was then turned on. If the Nellcor did not detect %Sp02 (saturation of hemoglobin with oxygen as measured by pulse oximetry) level or pulse rate, the PI improved the signal by:

- Securing the cable
- Securing the headband
- Warming the site
- Cleaning the site
- Repositioning the sensor
- Checking or changing the adhesive wrap
- Choosing an alternate site (above left eyebrow)
- Using another new adhesive forehead reflectance sensor
- Using the finger sensor

If any athletes appeared to desaturate (SpO2 ≤ 91%) the intent was to then later put them into a subgroup for future analysis.

Water Samples

Four samples (250 ml) of commercially bottled Activated Stabilized Oxygen® (ASO) (Bio2 International, San Luis Obispo, CA) oxygenated water and four samples of commercially bottled Aquafina ® (PepsiCo. Inc., United States) were randomly selected from approximately 48 bottles of water that were used in this study and analyzed for oxygen content. A Hach
Dissolved Oxygen Meter (HQ40D, Colorado) was used to determine the dissolved oxygen content of the water. The dissolved oxygen meter was calibrated prior to testing each water sample. All commercially bottled waters were tested immediately upon opening of the bottles. Both ASO and placebo waters were kept in the HPL in the research refrigerator at 42 °F, this temperature was recorded. Water samples were assigned by a third party. Prior to the subject drinking the water; the assigned water was poured into a 520 ml cup, so the subject would not know the type of water being consumed. After the initial 2-mile test, subjects were randomly assigned to either ASO water or bottled water (i.e., the placebo). On each day of the VO2max tests, the subjects drank 500 ml of their assigned water condition 15 minutes before running on the treadmill (Duncan, 1997; Jenkins et al., 2001; Mielke, 2004).

Statistical Analysis

Descriptive statistics were reported as mean ± SD. The matched (dependent) t-test was used to determine if there was significant differences in aerobic performance determined by: values of SpO2, HR, VO2, and RPE at the highest common workload achieved between the two tests for each athlete, as well as SpO2 at exhaustion, time to exhaustion, lowest SpO2 during the tests VO2max and Max HR under the conditions of ASO as compared to the condition of Aquafina® bottled water (placebo). The criterion for significance was set at an alpha level of \( p \leq .05 \). Statistics were analyzed using PASW Statistics (formally SPSS, IBM Corp., Chicago, IL, USA) version 17.0.2 with the criterion for significance set at an alpha level of \( p \leq .05 \).

Limitations

Although training was similar in the week preceding each of the two VO2max tests the workouts were not identical. The training and times for training for the weeks preceding each VO2max test was documented by the PI. The PI assumed that the athletes would replicate their
diet in the 36 hours prior to each of the VO$_2$max tests but no diet analysis was performed. Conditioning level and previous experience of the athletes were not controlled. The fatigue from previous workouts was presumed not to affect VO$_2$max testing. The athletes did report that ASO water also had a “salty” taste, where as the placebo did not.

Delimitations

Delimitations of the study that would affect the generalization of the study included: 1) subjects were college-aged males who were currently playing Division II soccer at Humboldt State University; 2) the researcher only measured SpO$_2$ during max test and not during the timed 2-mile run; 3) SpO$_2$ was measured using only Nellcor Oximax® N-560 Pulse Oximeter; 4) VO$_2$max was only assessed using the VO$_2$max Astrand and Rodahl Protocol and; 5) performance was only measured by time to exhaustion on an incremental treadmill protocol.

Operational Definitions

1) Off-season- As the period between the postseason and 6 weeks prior to the first contest of the next year’s season (Baechle, et al., 2008).

2) Oxyhemoglobin desaturation- Having a %SaO$_2$ value of $\leq$ 91% according to the oximeter, as was defined in studies by Dempsey (1984) and Miyachi et al. (1992).

3) Highest common workload (HCWL) – Is the highest workload the athlete achieved in the tests that was completed in both the place and ASO conditions.

4) Highest common workload (HCWL) - The highest workload the athlete achieved in the test that was completed in both the placebo and ASO conditions.

5) SpO$_2$- Saturation of hemoglobin with oxygen as measured by pulse oximetry and expressed as % (Shibata, Kawata, Miura, Shibata, Terao, & Sumikawa, (2002). SpO$_2$ was
recorded at the 2-sec value at the end of the HCWL stage and at the exhaustion point. The lowest \( \text{SpO}_2 \) value during the test was also recorded during the test.

6) \( \text{SaO}_2 \) - Arterial blood oxygen saturation (Shibata, et al., 2002)

7) \( \text{VO}_2 \) - Oxygen uptake by the body.

8) \( \text{VO}_{2\text{max}} \) - The maximal oxygen uptake or the maximum volume of oxygen that can be utilized in one minute during maximal or exhaustive exercise, measured as milliliters of oxygen used in one minute per kilogram of body weight (Williams, M., 2008).

9) Heart rate - The amount of beats per unit of time, usually measured in beat per minute (BPM). BPM was measured continuously throughout testing and taken as last stable HR at the HCWL and also as the highest HR achieved as measured using the Nellcor Oximax® N-560 Pulse Oximeter.

10) Borg Scale - A simple method of rating perceived exertion (RPE) used to gauge the athlete's level of intensity in training and competition. The scale ranged from 6 to 20. The participants were asked to use a thumbs up, down, or OK sign to acknowledge the overall level of how they were feeling every 15 seconds before the new stage started.
CHAPTER THREE

Results

This study was designed to compare aerobic endurance performance in Division II male soccer players under the conditions of Aquafina bottled water (placebo) and ASO. Participants in this study were the Humboldt State University Men’s Soccer team. In regards to soccer playing position, subjects represented multiple positions including a goalkeeper \((n = 1)\), defenders \((n = 4)\), midfielders \((n = 5)\), and forwards \((n = 2)\). It should be noted that some subjects reported to the experimental trials directly after their practice session, which could have skewed the results. These individuals however participated in the same amount of practice each time before coming to the trials.

Descriptive characteristics (including age, year of eligibility, weight, height, and 2-mile run times) for the 12 subjects who successfully completed the entire study are presented in Table 2. Descriptive statistics (mean ± SD) for the dependent variables for both the placebo and ASO condition are shown in Table 3.

Matched (dependent) t-tests were used to determine if there were significant differences at the highest common workload (HCWL) achieved in both conditions in \(\text{SpO}_2\), HR, \(\text{VO}_2\), and RPE (see Figures 1, 2, 3). Matched (dependent) t-tests were also used to determine if there were significant differences at exhaustion in \(\text{SpO}_2\) (Figure 4) and time (Figure 5) under the conditions of the placebo as compared to the condition of ASO. Matched t-tests were also utilized to determine if there were differences in mean \(\text{VO}_{2\text{max}}\) and HR maximum (see Figures 6 and 7). Finally, the lowest \(\text{SpO}_2\) value achieved during testing was compared between the two conditions; this generally occurred towards the last minute before exhaustion. Although there were no statistically significant differences found in mean values for any of the dependent
variables, there was an improvement in time to exhaustion in the ASO group by 23.34 seconds ($p = .072$). Finally, the values for oxygen content of the placebo water were consistently 2 mg/L. The ASO water had an oxygen concentration of $5.0 \pm 0.4$ mg/L of oxygen with a range of 5.2 to 4.8 mg/L of oxygen in the four samples analyzed.

Matched t-tests were also used to determine if there were significant differences between each level in VO$_2$, HR, VCO$_2$, and tidal volume (see Figures 8-11). Again there were no statistically significant differences found in mean values for any of the dependent variables.
Table 2

Subject Characteristics (N = 12).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
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<tr>
<td>Age (years)</td>
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<td>1.44</td>
<td>18.00</td>
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<tr>
<td>Eligibility (years)</td>
<td>2.58</td>
<td>1.31</td>
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<td>Weight (kg)</td>
<td>73.33</td>
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<td>Height (cm)</td>
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<td>166.00</td>
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<tr>
<td>2-mile run (sec)</td>
<td>754.08</td>
<td>19.36</td>
<td>713.00</td>
<td>781.00</td>
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</table>
Table 3

Mean Values for Dependent Variables Under Two Conditions (N = 12).

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>ASO</th>
<th>t (11)</th>
<th>p</th>
<th>95% CI</th>
<th>LL</th>
<th>UL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SpO₂ (HCWL)</td>
<td>95.58</td>
<td>3.05</td>
<td>96.08</td>
<td>2.46</td>
<td>-0.702</td>
<td>.497</td>
<td>-2.06</td>
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<tr>
<td>HR (HCWL)</td>
<td>187.00</td>
<td>8.66</td>
<td>187.16</td>
<td>7.63</td>
<td>-0.074</td>
<td>.942</td>
<td>-5.09</td>
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<tr>
<td>VO₂ (ml/kg/min) (HCWL)</td>
<td>57.26</td>
<td>4.93</td>
<td>58.15</td>
<td>3.87</td>
<td>-0.974</td>
<td>.351</td>
<td>-2.87</td>
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<tr>
<td>RPE (HCWL)</td>
<td>15.33</td>
<td>2.01</td>
<td>15.33</td>
<td>1.49</td>
<td>0.000</td>
<td>1.00</td>
<td>-1.21</td>
</tr>
<tr>
<td>SpO₂ at exhaustion</td>
<td>94.75</td>
<td>3.01</td>
<td>95.16</td>
<td>2.24</td>
<td>-0.526</td>
<td>.610</td>
<td>-2.16</td>
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<tr>
<td>Time to exhaustion (sec)</td>
<td>428.83</td>
<td>62.54</td>
<td>451.17</td>
<td>57.05</td>
<td>-1.99</td>
<td>.072</td>
<td>-46.98</td>
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<td>RPE at exhaustion</td>
<td>15.66</td>
<td>2.30</td>
<td>16.00</td>
<td>1.12</td>
<td>-0.561</td>
<td>.586</td>
<td>-1.64</td>
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<td>SpO₂ (lowest)</td>
<td>94.58</td>
<td>2.90</td>
<td>94.92</td>
<td>2.06</td>
<td>-0.352</td>
<td>.732</td>
<td>-2.00</td>
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<tr>
<td>VO₂ max (ml/kg/min)</td>
<td>59.97</td>
<td>4.24</td>
<td>60.92</td>
<td>3.51</td>
<td>-1.22</td>
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<td>Max HR</td>
<td>192.08</td>
<td>7.69</td>
<td>191.08</td>
<td>7.63</td>
<td>0.544</td>
<td>.597</td>
<td>-3.04</td>
</tr>
</tbody>
</table>

Note.; CI = Confidence interval of the difference; LL = lower limit; UL = upper limit; HCWL = highest common workload.
Figure 1. Comparison of SpO\textsubscript{2} at highest common workload between placebo group and ASO.
Figure 2. Comparison of HR at highest common workload between placebo group.
Figure 3. Comparison of VO$_2$ highest common workload between placebo group.
Figure 4. Comparison of SpO₂ between placebo group and ASO group at exhaustion.
Figure 5. Comparison of time to exhaustion between placebo group and ASO group.
Figure 6. Comparison of VO$_2$$_{max}$ achieved between the placebo group and ASO group.
Figure 7. Comparison of maximum HR between the placebo group and ASO group during treadmill testing procedures.
Figure 8. Comparison of VO$_2$ (ml/kg/min) between the placebo group and ASO group during treadmill testing procedures.
Figure 9. Comparison of heart rate (BPM) between the placebo group and ASO group during treadmill testing procedures.
Figure 10. Comparison of $\text{VCO}_2$ (L/min) between the placebo group and ASO group during treadmill testing procedures.
Figure 11. Comparison of tidal volume between the placebo group and ASO group during treadmill testing procedures. Vt= tidal volume
CHAPTER FOUR

Discussion

The purpose of the study was to determine if ASO would have an effect on aerobic endurance performance during maximal treadmill testing when compared to Aquafina® bottled water. The consumption of ASO resulted in no significant differences in the SpO₂, HR, VO₂, or RPE at the highest common work load achieved (HCWL) during the treadmill test compared to Aquafina® bottled water. Also, there were no any significant difference found in VO₂max or maximal HR between the ASO groups and the placebo condition. Finally, there were no statistically significant differences in SpO₂ at exhaustion, RPE at exhaustion, or time to exhaustion between conditions. While there was not any statistically significant differences concluded in mean values for any of the dependent variables, it should be noted that there was an improvement in time to exhaustion in the ASO group by 23.34 seconds ($p = .072$).

From previous studies we know that breathing in supplemental oxygen during exercise enhances performance (time to exhaustion) with an increased arterial oxygen content, decreased pulmonary ventilation, lower submaximal heart rate and blood lactate values, and a higher VO₂max (Bannister & Cunningham, 1954; Cunningham, 1966; Ekblom, Huot, Stein, & Thorstensson, 1975; Hughes, Clode, Edwards, Goodwin, & Jones, 1968; Dempsey 1986; Duncan, 1997; Hill, Long, & Lupton, 1924; Margaria, Edwards, & Dill, 1933; Miller, Perdue, Teague, & Ferbee, 1952; Tait, 1969; Welch, Mullin, & Welch, 1975; Welch, 1982; Wilson & Lewis 1974; Wilson, Wilson, Welch, & Liles, 1975). Although the breathing of supplemental oxygen during exercise has resulted in physiological and performance enhancement, the majority of studies using oxygenated water show no statistical evidence of enhancing sport or
physiological performance *during* exercise (Askew et al., 2000; Hampson et al., 2003; Leibetseder et al., 2006; Mielke, 2004; Willmert et al., 2001; Willmert et al., 2002; Wing, et al., 2003; Wing-Gaia, Sunbdhui, & Askew, 2005). That said, there are two studies in which oxygenated water was found to enhance performance. Jenkins et al. (2001) concluded that oxygenated water resulted in a higher percentage of oxygen saturation level at the end of the endurance test and at the end of a maximal exercise test compared to the placebo. Jenkins et al. (2001) also found that a subset of highly fit subjects with a VO$_{2\text{max}}$ of above 47 ml/kg/min had a greater time to exhaustion and higher SaO$_2$ level during maximal exercise testing after having consumed oxygenated water. Duncan (1997) concluded oxygenated water resulted in significantly faster 5K running time by an average of 31 seconds compared to the placebo condition.

A possibility of why oxygenated water may improve endurance performance is related to the idea of the hemoglobin desaturating. If an athlete desaturates or experiences exercise-induced hypoxemia, then supplemental oxygen may counter this effect and result in improved endurance performance. Exercise-induced hypoxemia has been thought to occur in athletes with high VO$_{2\text{max}}$ (Powers & Howley, 2007). There are only three studies, including the present study, in which SaO$_2$ values were measured when comparing the effect of oxygenated water to a placebo (Jenkins et al. 2001; Mielke, 2004). In the previously mentioned study by Jenkins et al. (2001) the consumption of oxygenated water resulted in a significantly higher SaO$_2$ value ($p > .05$) than the placebo. Mielke (2004) studied subjects whose VO$_{2\text{max}}$ averaged 54 ml/kg/min but failed to show significant differences in SaO$_2$ values at 60, 80 and 90% of their VO$_{2\text{max}}$ testing between the placebo and oxygenated water conditions. The subjects in the present study had an average VO$_{2\text{max}}$ of 59 ml/kg/min (range of 53.7 to 70.6 ml/kg/min) and did not show significantly higher
SpO₂ values at the HCWL or at exhaustion with ASO compared to the placebo. It should be noted that the oximeter in the current study had been malfunctioning and the data was not reliable.

The ability of oxygenated water to maintain or increase SpO₂ relative to a placebo would be dependent on how much oxygen was dissolved in the water. While Jenkins et al. (2001) did not report on oxygen content of the experimental water due to contract agreements with the company (T. Baynard, personal communication, Jan 10, 2010), five groups of researchers (Fuller [2010]; Hampson et al. [2003]; Leibetseder et al. [2006]; Mielke [2004]; Wilmert et al. [2001]) did test the oxygen content. All of the researchers who tested the oxygenated water found the oxygen content in the water was below the manufacturers claim. Wilmert et al. (2001) found the oxygen content in the oxygenized water was only 614.4 mg of oxygen per liter, while the bottled water had 217.6 mg of oxygen per liter. Hampson (2003) reported on five brands of oxygenated water. Four contained more oxygen than tap water, but the highest amount of oxygen content was 293.8 mg of oxygen per liter of water compared with 32.5 mg of oxygen per liter of water (Hampson, 2003). Mielke (2004) reported their oxygenated water (Life O₂) was significantly higher (~46% more oxygen) than non oxygenated water. Leibetseder (2006) also measured oxygen content in water and reported it contained 160 mg of oxygen per liter instead of 180 mg of oxygen per liter as the company claimed. The concentration also decreased to 74 mg of oxygen per liter stepwise after leaving the bottle open for 20 minutes and vortexing it in another bottle for 5 minutes. In the present study, the dissolved oxygen content in the ASO water and Aquafina® bottled water was measured. ASO was measured as having an average of 5.2 mg of oxygen per liter while Aquafina® averaged 2 mg of oxygen per liter. Although, the dissolved oxygen content in ASO was significantly higher than the bottled water, the dissolved oxygen
content was still very low compared to the manufacturer’s claim. Through personal communication from the manufacture, the correct way of measuring oxygen content in the ASO is “using an Oxidation-Reduction Potential (ORP) meter which will establish the mV level.”

Can the oxygen from the water get into the bloodstream and still be available to the working muscles? Cooper et al. (1960) and Forth and Adam (2001) agreed there is an increase of oxygen content in the portal vein. Forth and Adam reported after the administration of both the 80 and 150 mg of oxygen per liter in their water solutions, PO$_2$ in the portal vein was found to increase significantly by 14 mm Hg. No measures were made of systemic arterial oxygen content. Jenkins et al. (2001) found an increase in SaO$_2$ in their subject population, however, neither Meilke (2004) or the current investigator could conclude that the consumption of oxygenated water had a significant effect on SaO$_2$. Thus, it is still not clear as to whether the oxygenated water is able to get into the arterial blood system and working muscles.

While it appears doubtful that oxygenated water can increase aerobic performance from the multiple studies conducted, future research should follow up on the supplemental usage of ASO on athletes who desaturate in high intensity events. Future investigators should examine the quantity of ASO being ingested, as well as timing of when to consume ASO to help explain if the supplement will be able to prevent these athletes from desaturating. The ASO solution used in the current was “salty” tasting and could be detected by the subjects. Future researchers should attempt to mask the taste so the subject cannot distinguish the control from the experimental solution. Another important area of investigation would be measuring the blood oxygen saturation levels to explore the possibility of ASO entering the arterial blood stream. Also, the analysis of the oxygen content in the water should be measured appropriately. Lastly, it would be
of interest to examine ASO and its ability to increase time to exhaustion, as it has in other studies (Duncan, 1997; Jenkins et al., 2001).

In conclusion, there was no evidence in the current study that consuming ASO enhanced aerobic performance. One cause would be the malfunctioning oximeter during the trials, leaving the data unreliable. Although time to exhaustion was not significantly different between the placebo and ASO conditions, the time was extended 23.34 seconds ($p = .072$) more in the experimental group. Every second of additional endurance gained in an athlete’s event can make the difference between a victory or a loss. Further studies should be conducted to determine the potential effects ASO has on $\text{SaO}_2$ levels and aerobic performance.
REFERENCES


TM stabilized liquid oxygen as an ergogenic aid for sprinters in a competition/heat
scenario]. Unpublished raw data.
Participant Instructions

To: Research study participants
From: Peter Fuller

We appreciate your participation in this research study. By asking you to adhere to the conditions that follow, we are more likely to have results that we know are related to the water you ingest (oxygeated water or placebo) and not to your diet.

Instructions for participants before exercise testing to increase test validity and data accuracy. Please adhere to the following testing conditions:

- Do not eat one and a half hours prior to testing.
- Refrain from ingesting alcohol, caffeine, or smoking within 3 hours of testing.
- Keep track of their diet 36 hours prior to VO\textsubscript{2max} testing so it can be replicated 36 hours prior to the second VO\textsubscript{2max} testing.
- Rest for the assessments, avoiding significant exertion or exercise on the day of the VO\textsubscript{2max} and 2-mile run test.
- Your clothing should permit freedom of movement and include running shoes.
- Drink ample amount of fluid over 36 hour period preceding the test to ensure normal hydration before testing.
- Include ample amount of carbohydrates in your diet 36 hours prior to testing.
- Bring in dietary form on the day of your trials.

Test Date #1: ________________________________

Test Date #2: ________________________________

Time: ________________________________
APPENDIX A: Training Schedule of Athletes
APPENDIX A: Training Schedule of Athletes

HSU Men’s Soccer Training Schedule

Mondays: 60 minutes weights workout
         15 minutes team warm up
         40 minutes foot work drills

Tuesday: 10 minutes small group warm up
         50 minutes small group technical skills training session

Wednesday: 60 minutes weight workout
          15 minutes team warm up
          40 minutes team fitness

Thursday: 10 minutes small group warm up
          50 minutes small group technical skills training session

Friday: 60 minute weights workout
        60 minute unofficial captains practice - 6 v 6 games

This schedule is consistent (except for the occasional recruit visit / tryout) through the Spring Break. Once they return from Spring Break - Mondays and Wednesdays will stay the same and Tuesday, Thursday, Friday will be full team training for 2 hours through the end of March. In April Mondays will stay the same and they will have team training Tuesday-Friday. Fitness will mostly involve 12 - 15 minute timed fitness running drills followed by 10 - 15 minute sprint workouts. Occasionally they will have stadium stair runs or long distance track runs.
APPENDIX B: Informed Consent
APPENDIX B: Informed Consent

Can oxygenated water effect endurance performance in Division II male collegiate soccer players?

INFORMED CONSENT PARTICIPATION

Please read the following as it provides information about this research study. Please understand that you are being asked to volunteer in this study and it is your choice to participate. By signing this form you are indicating that you have been informed of the nature of the study including the risks and benefits of its association and want to participate.

You are being asked to participate in a research project by Peter Fuller, a graduate student in the Department of Kinesiology at Humboldt State University (1 Harpst Street, Arcata, CA). If there are any questions or concerns relating to this research please call Peter Fuller at (707) 362-0751 or email peterfuller11@gmail.com. You may also call or email the supervising faculty member Dr. Tina Manos at (707)826-5962, Tina.Manos@humboldt.edu. Additional contact may be made with the Dean of Research and Graduate Studies, Dr. Chris Hopper (707) 826-3853, cah3@humboldt.edu.

Explanation of Purpose
The primary purpose of this research is to determine if oxygenated water will increase SaO\textsubscript{2} levels, time to exhaustion and VO\textsubscript{2max} in male Division II soccer players. If the oxygenated water shows an improvement in these variables then this would have important implications for the training of endurance athletes.

Participants
Approximately 20 subjects from Humboldt State University Men’s soccer team will be recruited to participate in the study. Potential participants will be selected based on inclusion and exclusion criteria.

Procedures and Time Required
If you agree to participate in the study you will:
1. Complete a Medical Information and History questionnaire which determines the final inclusion into the study.
2. Have your height and weight measurements taken. These measurements will be taken by the principal investigator (Peter Fuller) in the Human Performance Lab to insure privacy.
3. During a day of your tryout you will perform a 2-mile run to qualify for the experimental trial. This procedure lasts approximately 40 minutes from warm-up to cool-down.
4. Upon qualification of the experimental trial, you will participate in two VO\textsubscript{2max} tests in the Human Performance Lab at HSU. This procedure will last approximately 50 minutes for each trial.
Location and approximate time commitment from participants (includes warm-up time for tests)
Introduction, signing of informed consent, demographics and dietary form
(Human Performance Laboratory)………………………………………………..…35 min
Two 2-mile timed run (HSU Redwood Bowl)………………………………….40 min
VO$_{2max}$ testing (Human Performance Laboratory)…………………………100 min
Total………………………………………………………………………………………..175 min

Description of Risks/Discomforts you may Experience and Risk Management Procedures

2-mile timed run
Risks and Discomforts: Risks include musculoskeletal injury.
Risk Management: You will be properly warmed up prior to performance.

Aerobic Fitness Test (VO$_{2max}$)
1. **Explanation of the Test.** You will perform an exercise test on a motor-driven treadmill. The exercise intensity will begin at a low level and will be advanced in stages depending on your fitness level. We may stop the test at any time because of signs of fatigue or changes in your heart rate. It is important for you to realize that you may stop when you wish because of feelings of fatigue or any other discomfort.
2. **Attendant Risk and Discomforts.** There exists the possibility of certain changes occurring during the test. They include abnormal blood pressure, fainting, irregular, fast or slow heart rhythm, and in rare instances, heart attack, stroke or death. Every effort will be made to minimize these risks by evaluation of preliminary information relating to your health and fitness and by observations during the testing. Emergency trained personnel are available to deal with unusual situations that may arise.
3. **Risk management.** You will have adequate time to warm up prior to performance.
4. **Responsibilities of the Participant.** Information you possess about your health status or previous experiences of unusual feelings with physical effort may affect the safety and value of your exercise test. Your prompt reporting of feelings during the exercise test itself is also of great importance. You are responsible for fully disclosing such information when requested by the testing staff.
5. **Benefits to be Expected.** By doing this research we are expanding the knowledge of oxygenated water and its effect on endurance performance. If we can improve oxygenation to the body in Division II men’s soccer players, we can push our bodies harder in endurance events. This will also help determine proper training methods to improve or maintain that level of endurance. Soccer coaches, strength and conditioning coaches and athletes alike will be able to design and implement training regimens. Subjects will be released the data from all tests and will benefit immediately from participation in this study as results will specifically inform them of their current aerobic physical capabilities.
6. **Inquiries.** Any questions about the procedures used in the exercise test or the results of your test are encouraged. If you have any concerns or questions, please ask us contact the primary investigator Peter Fuller at (707) 362-0751 or email at peterfuller11@gmail.com.
7. **Freedom of consent.** Your permission to perform this exercise test is voluntary. You are free to stop the test at any point, if you so desire.
8. **Confidentiality.** All tests and accompanying records will remain private unless specifically released through client’s written consent.
9. **Compensation.** You will not be paid for participation in this research
HUMBOLDT STATE UNIVERSITY RELEASE OF LIABILITY, PROMISE NOT TO SUE, ASSUMPTION OF RISK AND AGREEMENT TO PAY CLAIMS

I have read this form, and I understand the test procedures that I will perform and the attendant risks and discomforts. Knowing these risks and discomforts, and having had an opportunity to ask questions that have been answered to my satisfaction, I consent to participate in this test.

In consideration for being allowed to participate in this Activity, on behalf of myself and my next of kin, heirs and representatives, I release from all liability and promise not to sue the State of California, the Trustees of The California State University, California State University, Humboldt State University and their employees, officers, directors, volunteers and agents (collectively “University”) from any and all claims, including claims of the University’s negligence, resulting in any physical or psychological injury (including paralysis and death), illness, damages, or economic or emotional loss I may suffer because of my participation in this Activity, including travel to, from and during the Activity.

I am voluntarily participating in this Activity. I am aware of the risks associated with traveling to/from and participating in this Activity, which include but are not limited to physical or psychological injury, pain, suffering, illness, disfigurement, temporary or permanent disability (including paralysis), economic or emotional loss, and/or death. I understand that these injuries or outcomes may arise from my own or other’s actions, inaction, or negligence; conditions related to travel; or the condition of the Activity location(s). Nonetheless, I assume all related risks, both known or unknown to me, of my participation in this Activity, including travel to, from and during the Activity.

I agree to hold the University harmless from any and all claims, including attorney’s fees or damage to my personal property that may occur as a result of my participation in this activity, including travel to, from and during the Activity. If the University incurs any of these types of expenses, I agree to reimburse the University. If I need medical treatment, I agree to be financially responsible for any costs incurred as a result of such treatment. I am aware and understand that I should carry my own health insurance.

Date: ______ Name of Subject (Printed)__________________________________________

Date:_______ Signature of Subject:______________________________________________

Date:_______ Signature of Witness:______________________________________________
APPENDIX C: Medical and History Questionnaire
APPENDIX C: Medical and History Questionnaire

Medical Information and History, Questionnaire
The privileged information that is obtained in this questionnaire will be treated at the utmost confidential level as described in Health Insurance Portability and Accountability Act of 1996. Other than the information that is used for statistical and scientific purposes, no personal information will be released to any person. If any questions should arise please feel free to contact the Principle Investigator, Peter Fuller (707) 362-075 or email peterfuller11@gmail.com.

General Information
Name __________________________________________  Participant #___
Address_________________________________________ (for PI use only)
Phone # _________________________________________
Age ___________________ Date of Birth__________ Gender________
Playing position (s) __________________________________________________
Year of eligibility ________________________________________________

Emergency Contact
Name: ____________________     Phone: ________________________
Student ( )  Staff/Faculty ( )  Community ( )  Athlete ( )

Exercise Patterns during current season.
Currently on average, how many days a week do you (please fill in the following, if none please indicate (O):
1. Play Soccer? __________
2. Lift Weights? ___________
3. Do plyometric training? _________
4. Run (more than 30 min.)?_______

It is very important you take the time to read the following medical information and history information as they may affect your testing progress in the study.

Medical History and Health Related Questions. In the past five years have you had: (check yes or no):

YES   NO
( ) ( ) 1. Do you have any musculoskeletal, orthopedic (muscle, joint, ligament) condition, or medical condition that is made worse by exercise?
( ) ( ) 2. Do you know of any reason why you should not participate in strenuous physical activity?
( ) ( ) 3. Pain or discomfort in chest, neck, jaw, or arms
( ) ( ) 4. Shortness of breath or difficulty breathing at rest or with mild exertion e.g., walking).
( ) ( ) 5. Dizziness or fainting
( ) ( ) 6. Ankle edema (swelling)
( ) ( ) 7. Heart palpitations (forceful or rapid beating of heart)
( ) ( ) 8. Pain, burning, or cramping in leg with walking
9. Heart murmur
10. Unusual fatigue with mild exertion

Have you ever had:

11. Heart disease, heart attack, and/or heart surgery
12. Abnormal EKG or rhythm disturbance
13. Stroke
15. Asthma or any other pulmonary (lung) condition
16. Heart or blood vessel abnormality (e.g., suspected or known aneurysm).
17. Liver or kidney disease
18. Thyroid disorder
19. Are you currently under the care of a physician?
20. Do you currently have an acute systemic infection, accompanied by a
21. Do you have a pacemaker or implanted/external cardiac defibrillator?
22. Seizures
23. Do you have a chronic infectious disease (e.g. mononucleosis, hepatitis, AIDS)?

If you answered yes to any of these questions, please explain.

____________________________________________________________________________________
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Risk Factors

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<tr>
<th>YES</th>
<th>NO</th>
<th>DON’T KNOW</th>
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<td>(   ) (   ) (   )</td>
<td>1. Did any immediate family member have a heart attack before the age of 65?</td>
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<td>2. Do you have a father or brother who had a heart attack or heart surgery before age 55?</td>
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<td>(   ) (   ) (   )</td>
<td>3. Do you have a mother or sister who had a heart attack before age 65?</td>
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<td>4. Do you smoke or have you quit in the past 6 months?</td>
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<td>(   ) (   ) (   )</td>
<td>5. Do you have high blood pressure (&gt;140/90mmHg)?</td>
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<td>6. Do you have high total cholesterol (&gt;200 mg/dL)?</td>
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<td>7. Do you have high LDL cholesterol (&gt;130 mg/dL)?</td>
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<td>8. Are you taking cholesterol lowering medication?</td>
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<td>9. Do you have low HDL cholesterol (&lt;40 mg/dL)?</td>
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<td>10. Is your HDL cholesterol &gt;60mg/dL?</td>
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<td>(   ) (   ) (   )</td>
<td>11. Is your fasting blood glucose &gt;100 mg/dL (i.e., are you</td>
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12. Do you exercise regularly? If so, describe:

- **Frequency:** _________ days/week
- **Average Workout Time:** ________ min.
- **Intensity (circle):** Low  Moderate  High
- **How many months have you been active:** _________

If you answered yes to any of these questions, please explain.

______________________________________________________________________________

______________________________________________________________________________

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______________________________________________________________________________

Health-Related Questions

**YES  NO**

( ) ( ) 1. Have you had any surgery, serious illness, or serious injury in the last two years?

( ) ( ) 2. Are allergic to isopropyl alcohol (rubbing alcohol)?

( ) ( ) 3. Do you have any allergies to medications, bees, foods, etc.?

( ) ( ) 4. Are you currently taking any medications, supplements, or pills? If so, please list on the next page.

( ) ( ) 5. Do you have any skin problems?

( ) ( ) 6. Do you have any other illness, disease, or medical condition (beyond those already covered in this questionnaire)?

( ) ( ) 7. Have you had any caffeine, food, or alcohol in the past 3 hours?

( ) ( ) 8. Have you exercised today?

( ) ( ) 9. Are you feeling well and healthy today?

( ) ( ) 10. Are you currently under the care of a physician?

If you answered yes to any of these questions, please explain.

______________________________________________________________________________

______________________________________________________________________________

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______________________________________________________________________________
**Medications**

Please Select Any Medications You Are Currently Using:

- □ Diuretics
- □ Beta Blockers
- □ Vasodilators
- □ Alpha Blockers
- □ Calcium Channel Blockers
- □ Other Cardiovascular
- □ NSAIDS/Anti-inflammatories (Motrin, Advil)
- □ Cholesterol
- □ Diabetes/Insulin
- □ Other Drugs (record below).

Please list your current medications and/or supplements here. Include dosage and frequency.

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<th>Medication</th>
<th>Dosage</th>
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I certify that the information I have provided is complete and accurate to the best of my knowledge.

Date __________ Signature of Subject ____________________________________________

Date __________ Signature of Witness ____________________________________________

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For Office Use Only

- ____ Low Risk
- ____ Moderate Risk
- ____ High Risk
APPENDIX D: Dietary Form
APPENDIX D: Dietary Form

**Dietary Form**

Name ___________________________________________  
Date/Time of 1st Treadmill Test ______________________  
Date/Time to start 1st Diet Record _____________________  

Please include any and all foods consumed on this day including beverages in ounces if possible. This diet will be repeated in seven days.

**36-Hour Diet Record Form.**

Fill out the following information as accurately as possible. In the 36 hours prior to the first VO$_{2\text{max}}$ treadmill test provide a record that reflects day-to-day variability in your food consumption. This diet should then be replicated 36 hours prior to the second VO$_{2\text{max}}$ treadmill test (i.e., same food, same beverages, same quantities, same time…as much as possible) to control for any dietary influences on test performance. Use the example given as a guide to develop your detailed food intake record. Use additional pages as necessary. It is important to return the information to Peter Fuller (707) 362-0751, peterfuller11@gmail.com. **Please remember not to eat or drink anything 1½ hours prior to testing.** Below is an example of how to record your 3-day diet.

| Day of the week, meal eaten, and time. | Food or beverage  
Give a detailed description including type of food, brand name or restaurant | Amount eaten  
Give as tsp, tbsp, cups, oz, weight or portion | How was it prepared?  
Provide description, product label or recipe if available | Added salt or sugar? |
|--------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------|
| Example:  
Mon 2/1  
Breakfast  
8am | Scrambled eggs | 1 whole large egg | Scrambled in 1tsp butter with 1 tbsp 1% milk | Dash of salt and pepper |
| Mon 2/1  
Breakfast  
8am | Bread with margarine and jelly | 1 slice  
1tsp of each | Toasted | 1 tsp butter; 1 tsp jelly |
| Mon 2/1  
Breakfast  
8am | Orange Juice | 16 oz | Orangina | 100% juice not sweetened |
| Mon 2/1  
Snack  
10am | 20 pretzels | 20 | | |
### Test # 1

| Day of the week meal eaten, and time | Food or beverage  
Give a detailed description including type of food, brand name or restaurant | Amount eaten  
Give as tsp, tbsp, cups, oz, weight or portion | How was it prepared?  
Provide description, product label or recipe if available | Added salt or sugar? |
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<th>Day of the week meal eaten, and time</th>
<th>Food or beverage Give a detailed description including type of food, brand name or restaurant</th>
<th>Amount eaten Give as tsp, tbsp, cups, oz, weight or portion</th>
<th>How was it prepared? Provide description, product label or recipe if available</th>
<th>Added salt or sugar?</th>
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APPENDIX E: Sample Data Collection Sheet
**APPENDIX E: Sample Data Collection Sheet**

**Sample Data Collection Sheet**

This area will be used by the Principal Investigator

| Name: ___________________________ | Date: __________________________ |
| Participant # __________ (For PI use only) | Blood Pressure: __________________ |
| Height (cm): ___________________________ | Weight (lbs): ______________________ |
| Humidity: ___________________________ | Temperature: ______________________ |
| VO$_{2\text{max}}$ (kg/ml/min): ___________________ | Average SpO$_2$: ___________________ |
| Highest SpO$_2$: ___________________ | Lowest SpO$_2$: ___________________ |
| Average HR: ___________________ | 2-mile run time: ____ min ____ sec (nearest second) |

1) Did you get a chance to practice on an inclined treadmill  Yes ___ No ____
2) (Ask only on 2$^{\text{nd}}$ visit) Did you adhere to diet replication  Yes ___ No ____ Somewhat ____

Treadmill start time: ________________ Treadmill end time: ________________

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Verbal Instructions for 2-mile run

2-mile run
1) Prior to run, subjects were instructed to run the test at a maximal effort since their times were used not only for the study but as part of their fitness for the day.
2) They were also instructed in a demonstration on how to listen for their time as the completed each lap.
3) After their 15 minute team warm-up the subjects were instructed to line up with preferred foot on starting line.
4) On the assistant coaches command “Go” participants would start their run.
5) As participants complete each lap the assistant coach would announce the time to the nearest second.
6) Participants were notified by the assistant coach when they are on their last lap as well as their time upon completing the 2-mile run.
7) The subjects were allowed a 5 minute rest period before continuing their fitness session.