Oxygen Saturation During a Simulation Dive Response: Development of a Protocol for an Undergraduate Physiology Laboratory

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Oxygen Saturation During a Simulation Dive Response: Development of a Protocol for an Undergraduate Physiology Laboratory

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Abstract
There is room for improvement in the amount of active learning that takes place during physiology laboratory exercises. This paper describes a protocol designed to examine the human dive response, with the goal of creating an integrative laboratory experienced focused around improving active learning. The objectives of this laboratory exercise were to promote critical thinking, maximize collaboration and student engagement, and meet core competencies set forth by the National Science Foundation. Suggested modifications for portions of the exercise that were not successful have been included. By incorporating these suggestions, this activity could become an exercise used to progress physiology education based on survey, discussion question, and dive response data collected. doi: 10.21692/haps.2017.054

Key words: active learning, physiology, laboratory education

The information contained in this article will enhance student comprehension of human physiology and their appreciation for data collection, analysis and interpretation and laboratory experimentation associated with the pedagogy of courses in Human Physiology, Physiology Laboratory, and Human Anatomy and Physiology courses.

Introduction
For over 100 years, the teaching laboratory has remained essential to education in the natural sciences. Successful educators use laboratory time to engage students outside of the traditional classroom, to boost both student knowledge and appreciation of the sciences (Hofstein and Lunetta, 2004). As classroom education is revamped and improved, so too must laboratory education (Wei, 2011).

A common problem in laboratory education is simply keeping students attentive. Laboratory activities are often done in groups and, in many cases, group activities allow for a few students to take the lead while one or two students sit quietly by, termed 'social loafing' (Michaelsen *et al*. 1997). Active learning is an instructional model that emphasizes the duty of learning on students. The goal is for students to be "doing things and thinking about the things they are doing." Moving from a lecture-centered environment to a student centered active learning classroom has been shown to increase student enjoyment and consequently improve knowledge retention (Silverthorn *et al*. 2006). A shift from The National Science Foundation's Vision and Change for Undergraduate Biology Education movement calls for more collaboration between students to enhance the learning environment (Wei 2011). Experiments work best when they engage students both physically and intellectually (Hofstein and Lunetta 2004).

Although active learning can be highly beneficial for learning and creating an engaging learning environment, there are barriers to its implementation. Instructors may have little time to restructure their current laboratory activities due to demanding schedules and numerous responsibilities (Silverthorn *et al*. 2006). Time constraints, coupled with limited resources available to collegiate laboratories, pose what seems to be a daunting task in improving education (Hofstein and Lunetta 2004). The result is a set of 'cook-book' laboratory experiments, where students rush through a protocol in order to finish quickly without thinking critically about the scientific concepts being demonstrated (Hofstein and Lunetta 2004).

Dive Response as an Undergraduate Laboratory Experiment
In physiology laboratories, it is not uncommon for students to participate in a mammalian dive response activity. The dive response is a reaction to apnea in cold water utilized by diving mammals to conserve oxygen (Gooden 1994). The medulla regulates breathing, heart rate, and blood pressure, requiring a constant supply of oxygen to keep a mammal alive. In times of oxygen deprivation, oxygen is shunted to vital organs to prolong life (Lindholm and Lundgren 2009). This response is a summation of three independent reflexes, which in unison have profound benefits (Panneton 2013). Breath hold diving triggers respiratory, cardiac, and vascular responses to aid in oxygen conservation (Gooden 1994). This reflex, along with other evolutionary adaptions, are vital to survival of diving mammals, which can spend up to 80% of their time underwater (Panneton 2013). While first studied in seals, the trait is believed to be conserved amongst all mammals as well as some birds (Panneton 2013). The classic manipulation of

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the dive response in a teaching laboratory revolves around the relationship between heart rate, peripheral circulation, and oxygen saturation. In times of apnea, or suspended breathing, the need for oxygen is always greater within the brain and heart than the peripheral tissues (Choate *et al*. 2014).

The backbone of this activity is the measurement of oxygen saturation, which is the amount of oxygen bound to hemoglobin in the body. Oxygen saturation decreases slower between breaths than during normal breathing, as the need to conserve oxygen is increased. Interestingly, during simulated dives using cold-water facial immersion, the decline is slowed significantly (Andersson *et al*. 2002). Peripheral vasoconstriction is one of the body's main reflexes applied to conserve oxygen (Foster and Sheel 2005). The extremities, where the primary organs are muscles and skin, are the first areas to experience decreased oxygen availability (Elsner *et al*. 1971). By constricting blood vessels in the arms and legs, blood flow is shunted away from the extremities and towards the torso and head where the oxygen demand is higher (Foster and Sheel 2005). Similar to oxygen consumption, this reflex of vasoconstriction is amplified during simulated dives (Elsner *et al*. 1971). In full body submersion dives the response was even greater, with peripheral circulation decreased to almost zero (Gooden 1994). During times of apnea, heart rate is also decreased in an effort to conserve oxygen. Out of water breath holds resulted in an average decreased heart rate by 10%, while the drop was more significant with facial immersion, resulting in an average heart rate decrease of 25% (Foster and Sheel 2005).

A New Laboratory Exercise

The dive response has been a useful scientific demonstration since the 1940's (Choate *et al*. 2014). Simulated dives are an easy and relatively safe way to 'trick' the body into conserving more oxygen and blood flow for the heart and brain. (Foster and Sheel 2005). The integration of external stimuli and internal stimuli make for a good physiology-teaching laboratory, with multiple variables able to be manipulated via varying stimuli (Choate *et al*. 2014). In this study, peripheral circulation and oxygen saturation responses were combined with typical heart rate responses to create an engaging and stimulating laboratory experiment for the undergraduate physiology student. Physical exercise was also incorporated into the experiment to add another physiological principle for comparison. The goal was to have multiple robust variables to create a truly integrative experiment.

In an attempt to address the National Science Foundation core competency of demonstrating a relationship between science and society (Wei 2011), information about human uses for the dive response was included in the information given to participants. In brief, there are multiple human athletic feats that can be credited to the dive response. Similar to diving mammals, Ama divers in Japan and Korea used the dive response for dives of up to 20 meters for approximately one minute in order to gather food resources (Lindhold and Lundgren 2009). In comparison, professional divers today are able to dive for up to ten minutes, due to the dive response and an acquired heightened tolerance to carbon dioxide levels (Ferretti 2001).

Methods

Participant Recruitment and information

Participants in this mock laboratory exercise were 18 University of Mississippi students, ten males and eight females. The students' ages ranged from 19 to 22. Participant educational backgrounds varied, but each of the six groups of three participants had at least one student who was an upper-class science major. This experiment was not part of any class at the university. All participants volunteered to be a part of this experiment, were healthy, and did not experience any injury or negative side effects because of this study. The Institutional Review Board, (IRB) #15x-026, approved the laboratory exercise protocol conducted as exempt and informed consent was obtained from all participants.

The protocol used in this experiment was designed to pose extremely low risk to participants while still keeping subjects physically active. Only one precaution involved in the dive response exercise recommended that subjects with extremely high levels of physical fitness not be chosen as a subject, as the simulated dive in cold-water could cause a low enough drop in heart rate to be dangerous for such individuals (Lindholm and Lundgren 2009). The other risk involved was due to the physical exercise asked of the students, running down five flights of stairs then back up to the laboratory classroom.

Two surveys were given to participants. The first survey was given at the beginning of the experiment, to be completed after participants read the background information and protocol. The second survey was given after the lab exercise was completed. Surveys were anonymous, and participants were given fifteen minutes to read the information and complete the pre-lab survey, while the post-lab survey took participants less than five minutes to complete.

Pre-Laboratory

Pre-lab surveys were given out to laboratory participants before beginning the dive response exercise (Table 1). This survey was given to measure participants' self-perceived understanding of the literature review that provided background information for the exercise, the purpose and intended results for the dive response, and the learning objectives for the lab. The last question of the survey also served as baseline data to measure the participants' enthusiasm towards completing the laboratory exercise. The surveys were Likert-style surveys and asked participants to give a rating from disagree to strongly agree in response to each statement (Fowler Jr. 2009). Chi-square analyses were performed for each question with four degrees of freedom, with the level of significance set at α=0.05 (Seigal and

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Castellan 1988). Chi-square analysis was used to compare participant responses to a normal distribution. These data along with the post-laboratory survey were used in determining success of the experiment as a teaching method.

Laboratory Exercise
The full student protocol is included in Appendix A (Student Handout). The relationships between the various apneas (out of water, with facial immersion, and out of water immediately after exercise) and ECG traces, as well as the relationship between the apneas and the amount of carbon dioxide present at exhalation were chosen for this experiment. Previous experimental results (Valic *et al*. 2006) were used to help predict and measure these variables in a three-hour lab period.

Dive Response Data Analyses
All dive response data were collected in LabChart 8.0.2 and analyzed in Microsoft Excel 2007. To measure cardiac contractions, the R-Wave amplitude was used and to measure heart rate the QT interval was used. Both measures were taken from the ECG trace produced by LabChart. The QT interval was chosen because it varies with heart rate. Peripheral oxygen saturation measured with the pulse oximeter gave data for lowest recorded oxygen saturation levels per apnea. The pulse trace was analyzed for pulse amplitude and pulse rate. Capnometry measured using a Vernier Carbon Dioxide Gas Sensor was used to analyze maximum amounts of carbon dioxide detected. Each subject entered the data gathered into an Excel spreadsheet for analysis and later submission at the end of the experiment. The data from all groups were compiled into one spreadsheet, and descriptive statistics (mean, minimum, maximum) for each variable were calculated. Additionally, a one-way analysis of variance (ANOVA) was used to test for significance of responses from the compiled data.

Post-Laboratory Survey
Post-laboratory surveys were given out to laboratory participants after completion of the dive response exercise and student analysis (Table 2). This survey was administered to measure participants' opinions of their engagement during the exercise, the quality of the protocol, satisfaction with the amount of time needed for completion of the exercise, knowledge gained, and thoughts on the usefulness for this exercise in physiology laboratories. The style of this survey was the same as the pre-laboratory survey, with the addition of asking students to rate the laboratory exercise on a scale of one to ten, with ten being the highest level of satisfaction, compared to previous laboratory exercises they had completed. Chi-square analyses for each question were carried out in the same manner as the pre-lab survey. The responses regarding enthusiasm on the pre-laboratory survey were compared to self-reported levels of engagement on the post-laboratory survey. In addition to the survey, the amount of knowledge gained by the students was measured via discussion questions in the experimental protocol.

Table 2. Chi-Square analysis of post-laboratory survey items

Statement	Strongly Agree	Somewhat Agree	Neutral	Somewhat Disagree	Disagree	Chi-Square Value
I thought the experiment was fun and engaging	11	4	1	1	1	20.89*
I was able to successfully complete the experiment within the 3-hour lab time frame	15	1	1	0	1	45.33*
I was able to understand the methods for analyzing and interpreting the data, and was able to understand the results	10	3	4	1	0	17*
I believe that the teaching objectives were met upon completion of the experiment	14	4	0	0	0	40.89*
I learned information through completing this experiment that will help me on other assessments in an upper division, physiology course	13	4	1	0	0	33.67*

* P-value < 0.01

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Results

Pre-Laboratory Survey Data

Chi-square analysis was utilized to assess the pre-laboratory survey responses. Chi-square values (Table 1) indicate all responses were significant at the threshold of $p<0.05$. The survey data showed that a greater number of participants felt they understood the information presented to them in the pre-laboratory setting than the number of students who indicated they were excited to complete the experiment.

Table 1. Chi-square analysis of pre-survey laboratory items

Statement	Strongly Agree	Somewhat Agree	Neutral	Somewhat Disagree	Disagree	Chi-Square Value
I found the literature review was easy to read and helpful for gaining some background knowledge for the experiment	12	5	1	0	0	29.22*
I understand the purpose of this experiment and the results I should observe	10	6	2	0	0	20.89*
I found the teaching objectives to be clearly stated	15	3	0	0	0	47*
I am excited to perform this experiment and expect it to be interesting and fun	9	4	4	1	0	13.67*

* P-value < 0.01

Dive Response Exercise Data

As shown in Figure 1, the average apnea time for each hold decreased significantly $p_{(2,15)}=0.00340$ across the conditions. After each apnea, subjects were asked to exhale into a plastic bag. Volume of exhaled air was measured with averages of 2.0 L without immersion, 2.6 L with cold-water immersion, and 2.2 L with exercise. Data showed no statistically significant difference in lung volume at $p_{(2,9)}=0.784$. The exhaled air was measured for carbon dioxide concentration, with an average of 1.39 V after non-immersed breath hold. This was used as the baseline voltage for the 50,000 ppm of carbon dioxide present at the end of an average human expiration. Using two-point calibration technique, ppm of carbon dioxide was estimated for the other two apneas. The exhalation after cold-water immersion had the lowest concentration of carbon dioxide with a calculated 47,842 ppm, while the exhalation following the post-physical exercise apnea had the highest above average concentration of carbon dioxide with a calculated 51,799 ppm. These differences between the various apneas were not significant at $p_{(2,14)}=0.968$ (Figure 3).

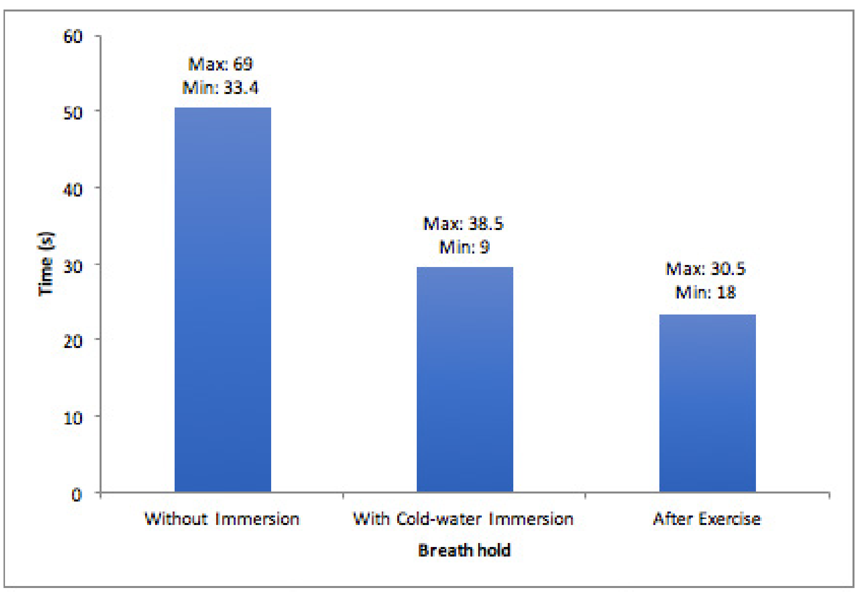

Figure 1. Average length of voluntary apnea under three different laboratory conditions. $P_{(2,15)}=0.0034$.

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Oxygen saturation in the subjects' peripheral systemic circulation was measured during each breath hold as well as during baseline testing (Figure 2). Average oxygen saturation was lowest during apnea with immersion (96.67%) and highest during baseline testing (97.47%). Nonetheless, the differences were not significant at $p_{(2,14)}=0.525$.

Pulse amplitude changed with each apnea, from an average of 44.14 mV at baseline to an average of 66.64 mV after exercise. The average for pulse amplitude without immersion was 56.40 mV and with cold water immersion was 57.48. These data for pulse amplitude are not significant at $p_{(2,9)}=0.104$. Pulse rate significantly changed from a high of 87.18 bpm during baseline testing to a low of 73.94 bpm during apnea without immersion, 66.85 bpm during apnea with immersion, $p_{(2,9)}=0.00652$ (Figure 3).

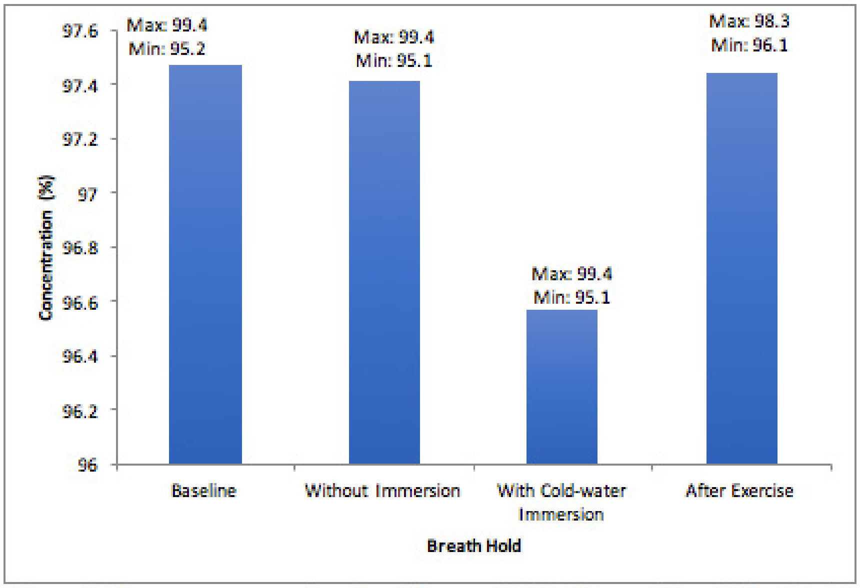

Figure 2. Average oxygen saturation of hemoglobin in the peripheral of systemic circulation of human subjects during apnea under 3 different laboratory conditions and baseline testing. $P_{(2,14)}=0.525$.

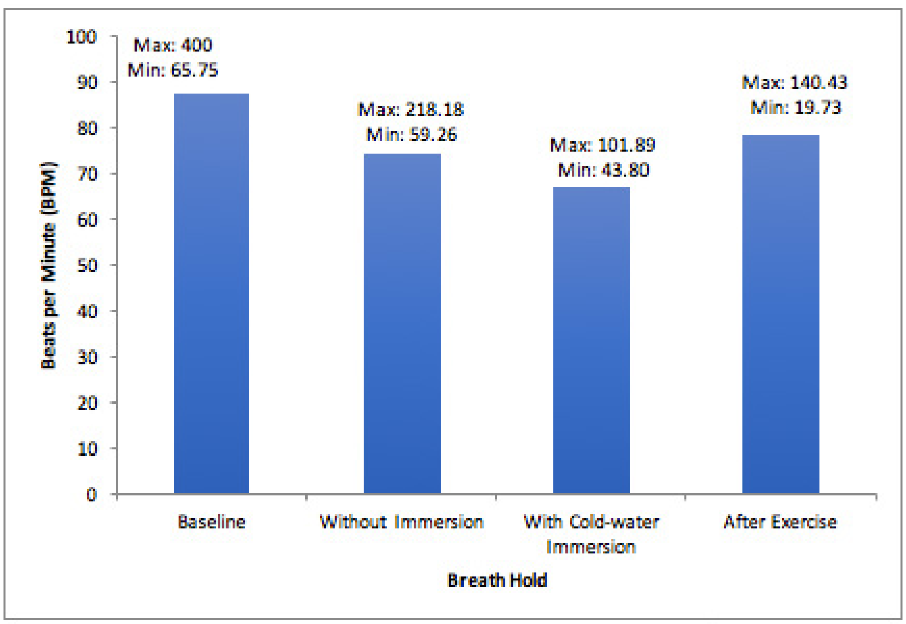

Figure 3. Average pulse rate during voluntary apnea under 3 different laboratory conditions and baseline testing. $P_{(2,9)}=0.0065$.

Amplitude of R-waves changed from a high of 0.77 mV during baseline testing to 0.56 mV during apnea without immersion, a low of 0.401 mV during apnea with cold water immersion, and 0.57 mV during apnea after exercise. This data was not significant, at $p_{(2,15)}=0.39$. Average QT interval changed from a low of 0.22 seconds during apnea after physical exercise to a high of 0.31 seconds during apnea with cold-water immersion. Average baseline QT interval was 0.25 seconds and average QT interval during apnea without immersion was 0.30 seconds. This data did not meet the requirement for statistical significance, at $p_{(2,15)}=0.0656$.

Post-Laboratory Survey Data

After completion of the entire exercise, students were given a post-laboratory Likert survey. Chi-square analysis indicated all responses were significant at the threshold set of $\alpha<0.05$ (Table 2). The first statement, that the experiment was fun and engaging, was significant at $p<0.01$. The remaining four statements were significant at $p<0.001$. Nine students had indicated "strongly agree" to the pre-laboratory survey item "I am excited to perform this experiment..." Data show that eleven students answered "strongly agree" to the post-survey item, "I thought the experiment was fun and engaging." Students were asked to compare the experience with other laboratory experiences they have had, and the answers averaged to an 8.5 out of 10, with 10 being the highest. The lowest score given was a 5, corresponding to an average lab experience.

Discussion Questions

Participants were asked to answer open-ended discussion questions following the exercise (see Appendix A). The majority of the answers received were correct. All six groups were able to correctly demonstrate an understanding of the relationship between oxygen saturation and peripheral

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circulation. Five of the groups were able to correctly interpret the results seen on ECG. Four of the groups correctly answered questions comparing changes in oxygen saturation or pulse rate.

Discussion

Pre- and Post-Laboratory Survey Data

The pre-laboratory survey given out to students served as a method to measure perceived learning from the background information and protocol given prior to the experiment, as well as to gauge student excitement towards completing the task. Since students were asked to participate on a Saturday, there was reasonable expectation that excitement scores would be lower than observed. This may have been due to the fact that participants were purposely asked to participate, rather than chosen at random. The post-laboratory survey measured student enjoyment of the exercise. It was notable that there was in increase in number of students who enjoyed the experiment as compared to those who indicated they were excited before lab began. This was encouraging, as student engagement and enjoyment of the experiment was one of the main goals to increase active learning.

Most students indicated they were able to understand the data and interpret the results. This was tested further by asking participants to answer discussion questions. Responses to those questions varied, with the majority of groups giving correct answers. This is consistent with the post-lab survey data; as some participants did answer neutral to understanding the results, and one participant even chose slightly disagree. There was one group in particular that had the most difficulty utilizing the data measurement equipment. It is possible that with unclean data, this group had the most trouble understanding and articulating the full scope of the intended learning material. Most groups gave excellent answers to the discussion question asking why apnea is more difficult to maintain after physical exercise.

It would have been intriguing to ask students via discussion question what made this laboratory experience better than others in their opinion, as all volunteers had previous experience in a collegiate science laboratory of some kind. It is possible that participants preferred this laboratory exercise because of the nature of the experiment, which helped keep students engaged by incorporating physical activity into the protocol.

Dive Response Data

Pulse rate decreased with apnea as expected, with a highly significant result (Foster and Sheel, 2005). Even with a small sample size, this result supports the successful didactic demonstration of the physiological phenomenon associated with the dive response. The length of each type of apnea was also significant ranging from 50.4 seconds (non-immersion) to 29.4 seconds (with immersion). These results are also typical of expected phenomena (Lindholm and Lundgren, 2009).

Carbon dioxide concentration in exhalations decreased after the cold-water immersion apnea and increased following the apnea after physical exercise. The decrease in carbon dioxide concentration after cold-water immersion could be due to subject discomfort in the cold-water leading to a premature exhalation when longer apnea could have been possible. One subject who was only able to hold his breath for nine seconds during cold-water immersion supports this claim.

While a drop in peripheral oxygen saturation was noticeable during the immersed dive as expected (Valic *et al*. 2006), the drop was too small to have statistical significance. The pulse oximeter used was of fairly high quality, giving readings in increments of 1%. The change in peripheral saturation was below instrument detection limits and unable to be effectively demonstrated and measured under these experimental conditions.

Lung volume and carbon dioxide concentration measurements were difficult to obtain. Of the six groups, only four were able to successfully capture exhaled air in the bags provided. Additionally, data collected from the four groups were affected by escape of expired air. The volumes of exhaled air were almost always well below the expected amount, based on typical vital capacities (Sherwood 2013). This flawed result was likely due to the extreme need for subjects to inhale following the apneas. The bags given to participants were cumbersome, being small in diameter and long in length. Possibly due to humidity in the classroom, each bag was stuck to itself and difficult to open in order to make room for the exhaled air. In a subsequent experiment, it would be recommended to try the manufactures Method 3 for data collection, exhaling through plastic straws into a bag already containing the capnometer.

Operator error with pulse transducers potentially led to non-significant data for pulse amplitude. Student difficulty keeping the transducer in place, as well as issues limiting arm and hand movements from affecting data were a challenge. While emphasized in the protocol and in the laboratory introduction, keeping the transducer attached at the appropriate tightness was a difficult task for subjects. Tighter pulse transducers led to extremely high pulse amplitudes, while loose pulse transducers led to extremely low pulse amplitudes, though the pulse itself is still detected under either condition. ECG trace data were not significant, but trends were observed. As pulse rate decreased in the apneas, QT intervals increased as would be expected (Sherwood 2013). Statistical significance for QT interval may have been able to be achieved with a larger sample size.

Suggested Modifications

The student data show that the main physiological phenomena of the dive response was successful, in that heart rate dropped during cold-water facial immersion and peripheral oxygen saturation decreased during cold-water

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immersion, albeit to differing levels of significance. In order to improve the exercise, a few changes suggested by students including the following:

- *A live demonstration in addition to written instructions would help make things clear.*
- *Breathing into the bags to measure carbon dioxide content was difficult to measure.*

Exhaling into a plastic bag to measure carbon dioxide concentration and lung volume was included in the experiment to highlight the fact that it is high carbon dioxide concentration in the cerebrospinal fluid, not low oxygen levels, that require a new breath (Young *et al.* 2013). Unfortunately, the process of measuring carbon dioxide concentration and volume of air expired was logistically problematic. Improvements to the protocol highlighting the importance of this task, as well as a classroom demonstration would likely be beneficial for future instruction.

A goal of this experiment was to meet as many of the core competencies outlined by the National Science Foundation as possible. These competencies are:

1) The ability to apply the process of science.
2) The ability to use quantitative reasoning.
3) The ability to use modeling and simulation.
4) The ability to tap into the interdisciplinary nature of science.
5) The ability to communicate and collaborate with other disciplines.
6) The ability to understand relationships between science and society.

(Wei 2011)

Through completing this exercise and making the observations in the discussion questions, participants were able to demonstrate the first competency. The second competency, using quantitative reasoning, was attempted through the data analysis portion of the lab. By linking the different variables together, such as peripheral vasoconstriction and its effect on oxygen saturation body, students utilized the fourth competency. While students did not collaborate with other disciplines, they did collaborate with each other and remained engaged through the laboratory exercise. Through some of the background information provided, as well as the data showing that breath hold length was longer non-immersed in water than while immersed in water, the exercise attempted to expose students to the sixth competency as well.

This exercise was designed to minimalize social loafing, maximize collaboration and student engagement, and promote critical thinking. Through the survey data collected and discussion question answers, it was shown that participants were able, for the most part, to effectively learn the intended information. This dive response protocol, with some suggested modifications, is a possible exercise to serve as a way to progress physiology education. The lab was challenging for students, both physically and mentally. The dive response testing was different than common laboratory protocols, where activity levels are low and collaboration is less prominent. This removed students from their comfort zones quickly, and exposed them to a different learning style, which they indicated increased engagement compared to other laboratory exercises. Overall, this experiment was effective in promoting an active learning environment, and with a few modifications can provide an excellent laboratory experience at various levels of physiology education.

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Literature cited

Andersson JP, Linér MH, Rünow E, and Schagatay EK (2002) Diving response and arterial oxygen saturation during apnea and exercise in breath-hold divers. *Journal of Applied Physiology*. *93*(3): 882-886.

Choate JK, Denton KM, Evans RG, & Hodgson Y (2014) Using stimulation of the diving reflex in humans to teach integrative physiology. *Advances in Physiology Education*. 38(4): 355-365.

Elsner R, Gooden BA, and Robinson SM (1971) Arterial blood gas changes and the diving response in man. *Aust J Exp Biol Med Sci*. *49*(5): 435-444.

Ferretti G (2001) Extreme human breath-hold diving. *European Journal of Applied Physiology*. *84*(4): 254-271.

Foster GE, and Sheel AW (2005) The human diving response, its function, and its control. *Scandinavian journal of medicine & science in sports*. *15*(1): 3-12.

Fowler Jr. FJ (2009) Survey Research Methods 4th Edition (Sage Publications Inc). 87-111.

Gooden BA (1994) Mechanism of the human diving response. *Integrative Physiological and Behavioral Science*. 29(1): 6-16.

Hofstein A, and Lunetta VN (2004) The laboratory in science education: Foundations for the twenty-first century. *Science education*. *88*(1): 28-54.

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Lindholm P, and Lundgren CE (2009) The physiology and pathophysiology of human breath-hold diving. *Journal of Applied Physiology*.*106*(1): 284-292.

Michaelsen LK, Fink LD, and Knight A (1997) Designing effective group activities: Lessons for classroom teaching and faculty development. *To Improve The Academy*. 385. 373-397.

Panneton WM (2013) The mammalian diving response: an enigmatic reflex to preserve life?. *Physiology*. *28*(5): 284-297.

Siegal S and Castellan Jr. J (1988) Nonparameteric Statistics for the Behavioral Sciences (McGraw-Hill, Inc). p. 323.

Sherwood L (2013) Human Physiology form Cells to Systems, Eighth Edition (Cengage Learning). pp. 456-480.

Silverthorn DU, Thorn PM, and Svinicki MD (2006) It's difficult to change the way we teach: lessons from the Integrative Themes in Physiology curriculum module project. *Advances in Physiology Education*. *30*(4): 204-214.

Valic Z, Palada I, Bakovic D, Valic M, Mardesic-Brakus S, and Dujic Z (2006) Muscle oxygen supply during cold face immersion in breath-hold divers and controls. *Aviation, Space, and Environmental Medicine*. *77*(12): 1224-1229.

Wei C (2011) "Vision and Change in Undergraduate Biology" Initiative Charts New Path for College-level Biology. National Science Foundation.

Young A, Marik PE, Sibole S, Grooms D, & Levitov A (2013) Changes in end-tidal carbon dioxide and volumetric carbon dioxide as predictors of volume responsiveness in hemodynamically unstable patients. *Journal of Cardiothoracic and Vascular Anesthesia*. 27(4): 681-684.

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Appendix A

Oxygen Saturation and the Dive Response
Directions for Students

Introduction

In this lab you will be measuring cardiovascular and respiratory response variables brought on by a simulated dive response. These variables change in response to cold-water facial immersion on oxygen saturation and peripheral circulation. You will also be measuring end-tidal carbon dioxide concentration. With each of the tests, you will additionally measure the effect of exercise on variables.

Background

In times of apnea, a time of suspended breathing, the need for oxygen is always greater within the brain and heart than the peripheral tissues. The dive response is a series of reflexes that can be triggered to amplify the body's response to apnea. The reflex is an evolutionary product conserved in humans from diving mammals, although the reasoning for this is unclear. It is vital to mammals such as seals and walruses, which must dive for long periods of time. The dive response was discovered in the 1940's, and has been a useful scientific demonstration since. The dive response is easy to perform and much more effective in observing oxygen conservation than normal breath holds. It is a safe way of "tricking" the body into saving oxygen for the heart and brain, as will be explained below.

The brain, specifically the medulla of the brain stem, keeps the body's vital functions working properly. The medulla regulates breathing, heart rate, and blood pressure, requiring a constant supply of oxygen to keep a person alive. In times of oxygen deprivation, oxygen needs to be sent primarily to this region to prolong life. Lower levels of oxygen in the blood will lead to oxygen deprivation and potentially death

Oxygen saturation is a measure of the level of oxygen bound to the hemoglobin in the blood, and can be measured via a technique called pulse oximetry. This is primarily done noninvasively by placing a receptor on the forehead, earlobe, or finger. Most commonly the finger is used, because it is the easiest to measure and provides accurate results. Pulse oximeters are common in hospital settings as the little 'clothespin' on the tip of the patient's finger. Normal oxygen saturation levels are within 95-100%. Levels below 90% are considered hyopoxemic, and below 80% are dangerous and can result in compromised organ function.

When a person holds their breath, for any reason, one of the body's responses is to conserve the oxygen still in the blood and direct it towards the brain. Ultimately, less oxygen is used in the peripherals in order to keep saturation levels higher for brain and heart functioning. Interestingly, during simulated dives using cold-water facial immersion, the drop in oxygen saturation is slowed even more. This means oxygen left in the blood when a breath hold begins lasts even longer when the face is immersed in cold water as compared to air. In the case of a drowning accident, a person who was drowning in ice-cold water would have a few minutes longer to be saved and revived before brain damage set in than in warm water or a person suffocating on land.

In order to conserve oxygen and save what remains for the brain, oxygen exchange must be slowed in other parts of the body. The extremities, where the primary organs are muscles and skin, are the first areas to experience decreased oxygen availability. By constricting blood vessels in the arms and legs, blood flow is shunted away from the extremities and towards the torso and head where oxygen is needed more. This action is called peripheral vasoconstriction. The amount of blood going to the arms in times of normal breathing versus times of breath holds will be measured using a pulse transducer. In full body submersion dives, such as would be the case in the drowning example, the response would be even greater with peripheral circulation decreased to almost nothing.

Against what may be popular belief, the physiological response that forces a new inhale is actually high carbon dioxide levels in the blood, not low oxygen levels. Carbon dioxide is a toxic gas if levels in the body become too high. While some of the toxicity is controlled within the blood by the body's natural buffer systems and diffusion through the skin, the rest needs to leave via exhalation. Basal carbon dioxide pressures in the body are about 40-46 mmHg systemically and about 25 mmHg in the cerebrospinal fluid (CSF). When carbon dioxide levels get too high in the CSF that surrounds the brain, the pH becomes too low. As a result, chemoreceptors within medulla are triggered. Consequently, we feel the need to exhale. While chemoreceptors for oxygen levels exist in the aorta and carotid artery, they are not stimulated until after carbon dioxide chemoreceptors have already signaled for a new breath. Exercise causes a rise in lactic acid, which will also lower blood pH. This is part of why we need to breathe more often when we exercise.

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In our experiment, we will be measuring carbon dioxide volumes at the end of each breath hold using a capnometer, a device that determines carbon dioxide content of gas. Responses to carbon dioxide levels vary between individuals, and this experiment will be an interesting way to determine a class average as well as distinguish how activity levels affect end-tidal carbon dioxide levels.

Learning Objectives

1. To learn and understand the causes of autonomic breathing, and the effect of exercise on breathing.
2. To measure the amount of carbon dioxide exhaled after a full apnea, and calculate and interpret class averages.
3. To analyze the effect of apnea on peripheral circulation and oxygen saturation in the blood.
4. To observe the effect of exercise on peripheral circulation and oxygen saturation in the blood.

Equipment List

- PowerLab 26T
- Pulse transducer
- Pulse oximeter
  - o Oxygen saturation meter
  - o Oximeter pod
- BioAmp cable
- 3 electrode leads
- 3 disposable electrode pads
- Abrasive pad
- Alcohol wipes
- Electrode gel
- 3 lung volume bags
- Carbon dioxide sensor (to be obtained from the front cart when available)
- Dishpan containing ice water
- Paper towels
- Goggles
- Scissors
- 6 Rubber bands

Protocol

1. Be sure to read all steps and sub-steps before beginning each step.
2. Participants will work in groups of three, with each student assuming a role of equipment operator, subject, or attendant. The equipment operator will work the PowerLab equipment and control the computer. The subject will be the one completing the apneas and exercise. The attendant will help the subject and equipment operator as necessary to ensure the experiment goes smoothly.
3. The attendant will prepare basin of cold water and ice, approximately 10-12 degrees C.
4. The equipment operator will turn on the computer, turn on the PowerLab 26T, and open up LabChart. Open up the settings file named "Oxygen Saturation and Dive Response".
5. The equipment operator will set up subject with ECG, pulse oximeter, and pulse transducer.
   a. Plug the BioAmp cable into the BioAmp port, occupying inputs 3&4.
   b. Install 3 electrode leads into the end of the BioAmp cable.
      i. The black lead should go into the port for channel 1 positive, the white lead for channel 1 negative, and the green lead for the ground.
   c. The attendant will place disposable electrode pads on inside of each wrist and one electrode pad on inside of ankle, with the positive and negative on each wrist, with the ground/Earth wire on ankle.
      i. Prepare skin by gently rubbing with the abrasive pad to remove dead skin, then clean area with alcohol wipes.
      ii. Apply a small amount of electrode gel to each area, and attach sticky disposable electrode pads.
   d. Insert the DIN connector from the pulse oximeter into input 1.
   e. The subject should place the pulse oximeter with the laser side facing the bottom of the index finger.
   f. Insert the DIN connector for the pulse transducer in input 2.
   g. The subject should wear the pulse transducer on the opposite hand middle finger, with the gel end firmly strapped to the pad of the finger.
6. The attendant will label 3 plastic bags for exhalation, labeled 1, 2, and 3. Using the scissors, cut the bags where at the 5 liter line.

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7. To gather baseline data, the equipment operator will click **start** and the subject will sit still and breathe normally. Gather data for 10 seconds, add a comment of baseline, then click **stop**.
8. The subject will perform the first breath hold out of water.
   a. Aim for 30 seconds or as long as possible. During the breath hold, the subject should focus on being as still as possible, as movements will affect the accuracy of the data gathered.
   b. The equipment operator will record data throughout the hold, entering a comment for breath hold 1 and another comment after exhalation. Click stop to end recording data 5 seconds after exhalation.
      i. To enter a comment, type into the text box below the graphs at the bottom of the window, then press return/enter.
   c. The subject will immediately breathe out the first breath into a plastic bag labeled 1. The bag will be tied off with a rubber band, preventing any air from escaping.
9. In order to better interpret data, the equipment operator should click AutoScale, which can be done at any time during the data recording process.
10. After a short rest, the attendant will put goggles on the subject. The subject will perform the second breath hold with the subjects face immersed in cold water. Again, aim for 30 seconds or **as long as possible**, monitored by the attendant. During the breath hold, the subject should focus on being as still as possible, as movements will affect the accuracy of the data gathered.
    a. The equipment operator will record data throughout the hold, entering a comment for breath hold 2 and another comment after exhalation. Click stop to end recording data 5 seconds after exhalation.
    b. The subject will immediately breathe out the first breath into a plastic bag labeled 2. The bag will be tied off with a rubber band, preventing any air from escaping.
11. In order to better interpret data, the equipment operator should click AutoScale, which can be done at any time during the data recording process. After completion of the second breath hold, the attendant may remove goggles from the subject.
12. Disconnect the DIN cables and bioAmp directly from PowerLab to be held by subject. The cables will be held by the subject. Electrode leads, pulse oximeter, and pulse transducer will remain in place on the subject. The subject will exercise by running from the top floor to bottom floor, and then back to top floor into the lab (five flights of stairs).
13. The subject will perform the final breath hold out of water. Aim for 30 seconds or **as long as possible**, monitored by the attendant. During the breath hold, the subject should focus on being as still as possible, as movements will affect the accuracy of the data gathered.
    a. The equipment operator will record data throughout the hold, entering a comment for breath hold 3 and another comment after exhalation. Click stop to end recording data 5 seconds after exhalation.
    b. The subject will immediately breathe out the first breath into a plastic bag labeled 3. The bag will be tied off with a rubber band, preventing any air from escaping.
14. In order to better interpret data, the equipment operator should click AutoScale, which can be done at any time during the data recording process.
15. Save the file with the title "Dive Response Lab" with group member's initials, for use later.
16. Once all the data has been collected from the pulse oximeter, the equipment operator will replace input one with the capnometer plug-in.
17. The equipment operator will close out of the current LabChart and open a new settings file labeled "Carbon Dioxide Concentration"
18. The lab instructor will perform a demonstration on how to use the capnometer correctly.
19. The attendant will place the capnometer into bag 1, with care taken to **prevent any air from escaping**. The capnometer may take up to 90 seconds to reach maximum carbon dioxide levels. Squeeze the bag as needed to ensure the capnometer has access to all the carbon dioxide in the bag.
20. The attendant will place the capnometer into bag 2, with care taken to **prevent any air from escaping**. The capnometer may take up to 90 seconds to reach maximum carbon dioxide levels. Squeeze the bag as needed to ensure the capnometer has access to all the carbon dioxide in the bag.
21. The attendant will place the capnometer into bag 3, with care taken to **prevent any air from escaping**. The capnometer may take up to 90 seconds to reach maximum carbon dioxide levels. Squeeze the bag as needed to ensure the capnometer has access to all the carbon dioxide in the bag.
22. Make sure to save this file, using the name "Capnometer Data" with group member's initials.
23. The equipment operator will then re-open the data gathered in the first part of the experiment.
24. The equipment operator will open the Excel spreadsheet entitled "Dive Response Data Collection".

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25. To begin, fill in the background information for the subject.
    a. Initials
    b. Gender
    c. Age
    d. Height
    e. Weight
    f. General activity level
26. For each breath hold and the baseline, the following data should be gathered and entered into the Excel table. The cursor and marker function of LabChart will need to be used. Please ask for assistance if it is needed.
    a. Length of breath hold (sec)
    b. Volume of exhaled air (L)
    c. Lowest oxygen saturation level (%)
    d. Pulse amplitude
    e. Pulse rate (bpm)
    f. R-wave amplitude
    g. QT interval (sec)
    h. Maximum carbon dioxide concentration (%)
27. The attendants for each group will write down the oxygen saturation values on the whiteboard. This data will be used to create second table in Excel.
28. The equipment operator will complete an ANOVA analysis of the data collected in the second table
    a. To do this, on the data tab click data analysis and then select Anova: Single Factor.
29. After the experiment and data analysis is completed, save the Excel spreadsheet as 'Dive Response Data Collection" with the group member's initials. Students will then answer the post lab questions.
30. Email the two data sheets from LabChart and the Excel data sheet to your instructor with the group member's initials as the subject line.

Post Lab Questions

Answer the following questions as a group, and write the answers out on a separate sheet of paper.

1. Had the final breath hold been performed with facial immersion, what results would you expect in regard to oxygen saturation and peripheral circulation?
2. How are peripheral circulation and overall blood oxygen saturation linked?
3. During the second breath hold (facial immersion), how did pulse rate compare to initial apnea and the baseline readings?
4. During the final breath hold, how did oxygen saturation compare to the previous breath holds?
5. Holding your breath becomes more difficult after the exercise in the final breath hold. Why is this?
6. How was the R wave amplitude affected by exercise? By the breath hold with facial immersion?
7. How was the QT interval affected by each breath hold?
8. After the completion of the Anova analysis, was the data deemed significant? ($p<0.05$)
9. What steps need clearer instructions?
10. What steps were already clear?
11. What suggestions do you have for making steps clearer?