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Abstract

Background and Purpose: Physiology exercise employing simulated diving is used in our curriculum to integrate knowledge in cardio-respiratory physiology. Aim was to improve model used in physiology exercise by employing continuous recordings of arterial pressure and heart rate.

Materials and Methods: Total of 55 medical and dental students volunteered for the exercise. They were instrumented with photoplethysmographic blood pressure and heart rate device, as well as with pulse oxymetry. Continuous measurement of variables was undertaken while students performed apneas or breathed through snorkel in air or in cold water, or temperature change was applied to their forehead.

Results: Employment of continuous recordings enabled detailed insight into changes in selected cardiovascular parameters during 30 seconds breathholding. Time course of the changes showed marked biphasic response. When face was submerged in cold water during apnea, arterial pressure initially decreased and heart rate increased. At the end of breath-hold, arterial pressure increased and heart rate decreased, respectively. Corresponding changes were less pronounced when breath-hold was performed without face immersion.

Conclusion: Improved protocol in laboratory exercise enabled us to show two distinct phases in changes of cardiovascular variables which are characteristic of diving reflex. We showed students how modern technology can improve their studies in near future and encouraged and motivate them to participate actively in exercise.

INTRODUCTION

Physiology education is present in the majority of biomedical science courses and diverse educational strategies have been used to improve the process of teaching and learning. However, to reduce overburdening of second year medical and dental students we propose an example of single physiology laboratory exercise to cover several major physiology topics with improving learning quality and reducing teaching hours. Excitement enhances the learning of any discipline, and physiology is no exception *(1)*.

Laboratory diving exercises have gained popularity as an investigative laboratory activity in medical curriculum. Diving and snorkeling are popular recreational and tourist activities with millions of people par-

ticipating in these activities each year (2). There are two types of diving - apnea or breath hold diving, in which the individual dives on their own held breath, and SCU-BA diving, where the individual uses a self contained underwater breathing apparatus filled with compressed air or some other gas mixture. Breath hold diving has long been a human activity, and recently amazing records have been recorded in this sport. Whether one is involved in world-record setting or just enjoying a diving vacation, the human body's response to breath holding and facial immersion in water is uniform and is termed the "diving response" (3, 4). Relatively simple acts of breath holding and forehead immersion in cold water can produce great changes in the cardiovascular system as "diving response". These changes are easily monitored and recorded, and thus can be used in simple physiology exercises to explain basic principles of the human physiology to students.

We propose employing photoplethysmography to continuously measure arterial blood pressure in physiology exercises. From such measurements we are able to calculate continuous beat-by-beat recordings of mean arterial pressure (MAP) and heart rate (HR). Using this technology we have been able to significantly improve the simulated diving laboratory exercises described by Hiebert and Burch (5) and others (6) in which only HR was measured with no emphasis on continuous measures.

The main goal of this work was to present improved diving physiology exercise using continuous measurements. Second goal, was to record the changes in cardiovascular variables induced by breath-holding in various settings, in order to explain diving reflex to students. Lastly, we wanted to offer several points of discussion with students which were not elaborated in the past, but are of clinical relevance for future physicians or dentists.

METHODS

Ethics and sample

The institutional Research and Ethics Board of the University of Split School of Medicine approved all protocols employed in described exercise, which were performed as part of the University's medical and dental physiology course. Total of 55 students, aged 19-21 years, of both gender participated in the exercise. They were all without apparent signs of acute illness, reported healthy, and volunteered to participate.

Equipment and supplies

An examining bed, plastic basin with removable cover, 1 towel per student, ice, thermometer, large diameter plastic tubing to be used as a snorkel, removable mouthpieces, noseclips, 2 liter plastic storage bags, disinfection chemicals, photoplethysmograph (Finometer, Finapress Medical Systems, Arnhem, Netherlands), mercury sphygmomanometer, pulse oximeter (Poet II, Criticare Systems, Waukesha, Wisconsin, USA), a data acquisition system and software (PowerLab 16S and Chart 5.0 software, ADInstruments, Castle Hill, Australia), personal computer, data graphing and statistical analysis software (Sigma Plot 8, Systat Software Inc., San Jose, CA, USA).

Laboratory exercise procedures

Students were asked to evaluate the effects of apnea and temperature on HR and MAP through a series of paired experiments. The principal protocol used was as described by Hiebert and Burch (5) and the various experiments are given in Table 1. Experiment 1 demonstrates the effects of a simulated dive where the subject holds their breath in cold water, experiment 2 examines the independent effect of apnea, experiment 3 examines the independent effect of facial immersion, and experiment 4 looks at the isolated effect of temperature.

During the exercise students work in groups of up to six and take turns being the experimental subject. Each experimental subject lies prone on the examining bed. The other students help to conduct the exercises: place pneumatic cuff for photoplethysmograph around the middle finger of the right hand of experimental subject, place probe for pulse oximetry on the second or fourth finger of the right hand-next to the pneumatic cuff and connect analog outputs to a data acquisition system. Experimental subject's head is resting on a towel placed on top of the water filled covered plastic bin. Water temperature in the plastic basin was monitored with a thermometer. Regular tap water was used, which in Split, Croatia varies between 12° and 15° C. Before performing apneas, students were permitted to take up to two deeper breaths, but longer hyperventilation was not permitted. Breath holds were carried out with a deep but not a maximal inhalation. When immersion was part of the experiment, students were instructed to immerse their heads in the water-filled plastic basins until their chins and at least half of their forehead was submerged in water. Each test lasts 30 seconds, as this length of apnea is comfortable and attainable for the vast majority of subjects. Students were instructed to discontinue breath holding if any discomfort developed. The other students help to measure the time, place a noseclip, wipe the face, pass the snorkel and watch the monitor and recording of all data.

Between tests, several minutes were allowed to pass in order for HR and blood pressure to return to normal baseline values. If the diving reflex did not fully develop within the 30 seconds of testing, at the end of testing we allowed students to perform maximal breath hold with all of the parameters being recorded as well.

Some possible obstacles in performing test were entrance of water in the nose, mouth, or eyes, difficulty with noseclip removal, subject distraction such as outside noise or laughter, shortness of breath, and inadequate arterial blood pressure signal. If such events disrupted the test, the test was repeated after HR and blood pressure parameters returned to normal.

Experiment	Procedure no.	Procedure description	
Simulated dive	1a	1a Breathing in air	
	1b	Holding breath in cold water	
Holding breath	2a	2a Breathing in air	
(apnea)	2b	Holding breath in air	
-	3a	Breathing through snorkel in cold water	
	3b	Holding breath in cold water	
Facial immersion	4a	Breathing through snorkel in air	
	4b	Breathing through snorkel in cold water	
	5a	Holding breath in air	
	5b	Holding breath in cold water	
Temperature	6a	Warm pack on forehead	
	6b	Cold pack on forehead	

TABLE 1Design of procedures employed in exercise.

Blood pressure measurements

Blood pressure was measured with the appropriate sized pneumatic cuff, which was placed around the middle finger of the right hand, and was connected to a photoplethysmograph. The arm of each subject was kept at heart level throughout experiments. Analog output of the photoplethysmograph was connected to a data acquisition system and digitalized. Sampling rate was set to 100 Hz. All data was stored on a personal computer. From continuous measurement of blood pressure, real time calculations of MAP and HR were continuously displayed on the monitor and recorded. At the end of each experiment, photoplethysmographically measured blood pressure was calibrated with the sphygmomanometer.

Arterial oxygen saturation measurements

Arterial oxygen saturation (SaO₂) was measured continuously by pulse oximetry with the probe placed on the second or fourth finger of the right hand, next to the pneumatic cuff. Thus, the left hand of the student remained free to perform manipulations related to simulated diving (i.e. - placing a noseclip, wiping the face, etc.). Analog output was directed to a data acquisition system, digitalized, continuously displayed on the monitor and recorded.

Statistical analysis

All tests were performed in pairs. Their order was not randomized. This was done in order to simplify the procedures and to allow for relatively simple statistics. Single, paired t-tests, which are built into the graphing program, were used for statistical analysis. If a test procedure was missed, it was relatively easy to repeat it without getting into the problems of randomization. Analysis of biphasic response of MAP and HR in response to 30 second breath hold with face immersed in cold water was performed by use of one-way ANOVA. Statistical significance was set at P < 0.05. Data was analyzed off line, after completion of each experiment using Power Lab software. For procedures where breathing was calm over the 30 seconds of testing (e.g. breathing in air, procedures 1a, 2a, etc), blood pressure and HR data were averaged over that time. For procedures where a physiologic response was expected (e.g. during experimental manipulations, procedures 1b, 2b, etc), maximal or minimal values of blood pressure were manually selected from tracings. Given that apnea caused a biphasic response in both blood pressure and heart rate data during the first round of experiments, we later analyzed the data by manually selecting both maximal and minimal values of the initial response from tracings.

All data was entered into an Excel spreadsheet (Microsoft, Inc., USA) where means, standard deviations and standard errors were calculated. Summarized data were then transferred to a graphing program for basic statistics and graphing purposes.

RESULTS

All 55 subjects included in this study were second year medical and dental students, Caucasians, mean age of 20 \pm 0.3 years (mean \pm SD). There were 32 males and 23 females. All subjects were already familiar with recreational diving and were able to hold breath for the full 30 seconds during the experiments.

A typical digital display of recorded real time calculations of MAP and HR is presented in Figure 1. Arterial oxygen saturation did not change with 30-seconds of apnea in the majority of subjects, and thus these data are excluded from analysis and commentary.

Summary data for the effects of apnea and temperature on MAP and HR during all experimental procedures for all students (n=55) are presented in Table 2. MAP significantly increased in procedures that included apnea, apnea in cold water and breathing through snorkel in cold water. HR significantly decreased in procedures that included apnea, apnea in cold water and breathing through TABLE 2

Summarized changes in mean arterial pressure (MAP) and heart rate (HR) for three generations of medical students (n=55).

Experiment	Procedure no.	MAP (mean ± SD†, mmHg)	HR (mean ± SD, bpm)
Simulated dive	1a	89.9 (12.7)	91.8 (15.2)
	1b	103.8 (16.3)*	69.0 (16.1)*
Holding breath	2a	91.5 (10.8)	89.0 (14.1)
(apnea)	2b	98.6 (11.9)*	74.7 (14.6)*
	3a	92.2 (12.6)	85.5 (14.0)
	3b	99.7 (14.7)*	72.7 (13.0)*
Facial immersion	4a	91.7 (11.5)	84.8 (11.9)
	4b	89.9 (13.6)*	83.0 (13.4)
	5a	97.9 (15.1)	72.2 (13.9)
	5b	99.9 (18.3)	65.8 (12.4)*
Temperature	6a	89.7 (14.5)	82.3 (12.0)
	6b	89.9 (15.3)	81.5 (12.0)

* denotes P<0.05 between procedures a and b by using single paired t-test
† Abbreviations: SD – standard deviation, bpm – beats per minute

snorkel in cold water. There were no significant differences in MAP and HR in procedures that investigated the effects of temperature only.

The results of a group of 22 subjects (single generation) were analyzed to demonstrate biphasic response to breathhold in arterial pressure and heart rate. All of them were second year medical and dental students, Caucasians, mean age of 20 ± 0.1 years, 14 males and 8 females. The biphasic response of MAP and HR during apnea with cold face immersion in the single generation (n=22) group of students is presented in Figure 2. With initiation of apnea MAP decreased, but then increased by the end of the apnea. HR showed an inverse change.

DISCUSSION

Main finding of this work is that application of continuous measurement of selected cardiovascular variables (arterial pressure and heart rate) markedly improves quality of physiology exercises. Moreover, it can reveal hidden components of physiologically induced reactions, such as biphasic response present in diving reflex evoked by breath-hold in this exercise. Thus, students are given deeper understanding of underlining physiological processes.

Experimental laboratory exercises that involve students as both, subjects and investigators, have great potential to teach students physiological principles. In general, stu-

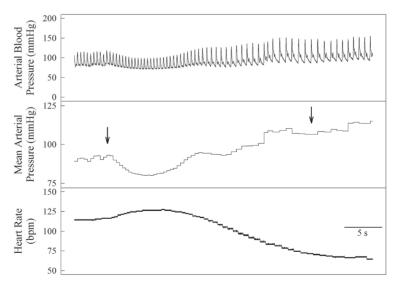
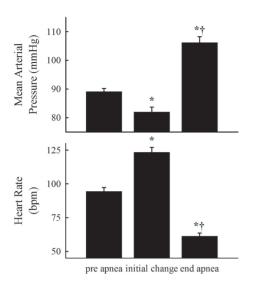
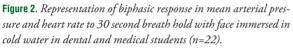


Figure 1. Response in arterial pressure (pulsatile and mean) and heart rate of one representative subject to 30 second breath hold with face immersed in cold water. At the beginning of breath hold (first arrow) there is a drop in arterial blood pressure and increase in heart rate, which is followed after ~10 seconds by opposite changes. Final increase in arterial blood pressure and decrease in heart rate remain changed for more than 5 seconds after termination of breath hold (second arrow), (bmp denotes beat per minute).





* Significantly different from pre apnea, P<0.05. † Significant difference between initial change and end apnea, P<0.05 by using one-way ANOVA.

dents enjoy participating in these physiology exercises and the exercises can open up lively discussion regarding other physiological issues. Given that the University of Split School of Medicine is located on the Adriatic Coast, most of our students have had personal experiences with breath-hold diving, and therefore, they were particularly curious about the physiology connected to this activity. In our physiology course, upon completion of the experimental exercises, we lead a discussion about human physiology and breath-hold diving.

TOPICS TO DISCUSS WITH STUDENTS Normal human heart rate and blood pressure

The ability to continuously measure variables and to display them readily to students is very important to their general understanding of physiology. When asked what the normal human heart rate is, the majority of students will answer 72 beats per minute, while a few might offer a range that is considered normal. Almost none will answer that it varies in the individual, between individuals, and that it varies in the same person depending on other variables, such as exercise (personal experience). The same holds true for blood pressure. Furthermore, measurements of arterial pressure in our experiments were more reliable and time aligned since we did not depend on student's ability to measure arterial pressure with mercury manometer at distinctive time points. Continuous measurement makes clear to students that MAP and HR both show great variability with various activities, such as breathing, speech, laughter, and limb movement. Another valuable lesson to be learned is that normal values quoted in textbooks are actually an average response obtained from many subjects and that individual reactions may deviate from what is expected.

Sympathetic and parasympathetic control of cardiovascular system – the diving response

The human body's response to breath holding and facial immersion in water is uniform and is termed the "diving response" (3, 4). The diving response is triggered by the immersion of the face in water or simply cooling of the face, which stimulates trigeminal receptors to send signals to the respiratory and cardiovascular centers in the medulla oblongata. These signals simultaneously activate the sympathetic and parasympathetic nervous systems. Sympathetic activation leads to peripheral vasoconstriction with subsequent hypertension and to constriction of the spleen, which increases the concentration of circulating hemoglobin. Parasympathetic activation leads to a decrease in HR and as a consequence decreased cardiac output. It takes 20-30 seconds to fully realize all of these changes (3, 4).

Regulation of breathing

Before leading into a discussion of the regulation of breathing, students are directed to answer the question, during a dive, how long do they stay at an achieved depth and why? In the beginning, students do not understand the point of this question since most of them do not linger for any significant time at a particular depth (they usually dive in, grab whatever they find at sea-bottom, and then rush to the surface). Next, we redirect them to describe the sensations they experience during the ascent phase of a dive. Some answer that they do not have enough air/oxygen to dive deeper and that they have great hunger for air/oxygen during surfacing. We also ask them how they prepare for a dive. Most answer that they take at least one deep breath, some take two, and quite a few answer more than a few. We ask the purpose of taking deep breaths before diving and the most common answer, even from experienced divers, is that they store more oxygen in the body that way.

Death of most experienced divers/ shallow water blackout

Unfortunately, hyperventilation before diving usually does not increase oxygen stores by more than 250 ml, but it does significantly decrease CO_2 (7, 8). This is an excelent opportunity to review the central control of breathing with students, pointing out the critical role that CO_2 accumulation has in triggering breathing. Hyperventilation before the apnea of diving represents the most dangerous maneuver a diver can make. It does allow divers to achieve

longer dives because it eliminates the trigger (CO_2) to breathe. Many experienced divers use this to their advantage, but how do they know how long they can stay underwater? For some divers, a decrease in blood oxygen tension can stimulate the need for a breath. Others rely on a watch to keep track of a known time before they lost consciousness in the past (7, 8).

Why is this so dangerous? The reason is very simple. According to Boyle's law diving to 10 meters (doubling the surrounding pressure) decreases lung volume roughly in half. Simultaneously, if one disregards O2 used for descent, the concentration or partial alveolar pressure of O₂ is doubled. A diver at that depth now has an abundance of O₂ without the protective mechanism of accumulating CO₂ which will urge him/her to breath (due to hyperventilation before dive) and he/she will stay under water longer than the critical time. In this case, the diver will be unable to consciousnessly reach surface. During the ascent from a prolonged dive, not only is that diver using O₂ in his lungs for the workout, but physiologic changes are working against the diver. During an ascent from 10 meters to the surface there is a doubling of lung volume, which thereby decreases the partial alveolar pressure of remaining O₂ in half. If it falls below 20-25 mmHg the diver will lose consciousness, stop ascending, and return to the bottom where he will eventually breathe in water and drown. Tragically, loss of consciousness usually occurs only a few meters below the surface. There are two additional factors that contribute to fatalities, especially among spearfishermen (8, 9). The first is that they usually dive alone, as a companion scare away fish. The second is that they keep their fishing spots secret from other divers. Hence, it is not uncommon that divers are alone the moment that they lose consciousness so that there is no one to pull them to the surface before they drown. So, the student may ask, why would a diver want to stay longer at the bottom when they are aware that it is so dangerous? Unfortunately, chasing a fish is not the best way to catch it. The free diver must patiently wait for the fish to approach him, and unfortunately, it can take a fish quite some time to swim toward him.

Limitations and problems

One limiting factor is that teaching outcomes of this peculiar exercise were not tested with appropriate or any specific questionnaire. However, official questionnaire performed by authorities revealed student's satisfaction with physiology course in general.

Two practical problems encountered during the exercises were that some students expressed fear about being unable to hold their breath for 30 seconds in front of colleagues (although this was rarely a problem) and many female students hesitated to immerse their faces in water because of they were wearing facial cosmetics. Both of these problems can be overcome easily with careful planning of the exercise. Announcing the exercise in advance and explaining what is to be expected usually leads to a sufficient number of volunteers for the experiments. As pointed out by Hiebert and Burch (5), if the course instructor volunteers as the first subject to be immersed in a bucket of water there is generally greater enthusiasm among students to participate themselves.

CONCLUSION

We described improvements in performing diving physiology exercise with medical and dental students and accentuated discussion points of diving medicine that can be made during and after exercise which are of interest of future medical and dental medicine doctors. This article presents an improved pedagogical method for teaching students in physiology course that is more enjoyable and attractive. This hands-on activity provides an effective educational tool to optimize knowledge acquisition in cardiovascular physiology by medical and dental students. Moreover, diving represents a worldwide popular human recreational activity and currently is target of many research groups. Thus, the development of pedagogical strategies to improve the learning and understanding of diving physiology would provide an important tool for teaching physiology in biomedical courses.

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