APNEA TRAINING AND PHYSICAL CHARACTERISTICS: ENHANCEMENT OF THE DIVE RESPONSE, APNEIC TIME, AND RECOVERY

by

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The following individuals read and discussed the thesis submitted by student Nathanael Stanford, and they evaluated his presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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ABSTRACT

Human breath-hold diving (free diving) has been gaining popularity as new individuals participate in the sport every year. Carbon dioxide (CO_2) breath-hold tables have been widely implemented as a training modality and prescribe constant submaximal apneic durations with gradually decreasing recovery times to increase hypercapnic resiliency. Previous studies implementing maximal duration apnea training have found enhancements in apneic duration and the mammalian dive reflex (MDR), which is elicited by apnea and augmented with facial immersion. The MDR results in bradycardia and peripheral vasoconstriction to redistribute blood to vital organs and elicit oxygen (O_2) conserving resulting in prolonged apneas. **PURPOSE:** The purpose of this study was to determine if CO_2 training tables are an effective means of enhancing apneic duration, the MDR, and recovery in untrained individuals, as well as determine if physical attributes affect differences in apnea training improvements. **HYPOTHESIS:** After apnea training, it was expected that participants would have an increased apneic duration, accentuated MDR, and faster recovery. Also, participants with a higher maximum O_2 consumption ($\dot{V}O_2max$), lean body mass percentage, and forced vital capacity (FVC) would benefit more from the apnea intervention. **METHODS:** 15 healthy participants with no previous breath-holding experience completed the training program (9 males, 6 females (22.67 ± 4.85 years of age)). After initial testing of physical characteristics, participants completed a dry apnea and 3 facially immersed (FI) static apneas. Arterial O_2 saturation (S_aO_2), heart rate (HR), mean arterial pressure (MAP), fraction of CO_2 in expired air ($FECO_2$), and fraction of O_2 in expired air (FEO_2) were used to assess the MDR and recovery. Participants then completed a 2-week apnea training intervention. This was followed by a post-test and a series of apneas matching the same duration as the apneas completed during the pre-test (time-mimicked (TM)). **STATISTICS:** Repeated measures ANOVA and paired t-test were used to determine if the MDR and apneic duration were enhanced. Pearson correlation coefficient analysis was used to determine if there was a relationship between physiological characteristics and improvements in the MDR and apneic duration. **RESULTS:** The easy-going phase was prolonged during the post and TM tests ($p<0.01$). There was no difference after training in the rate of onset and magnitude of bradycardia or differences in MAP. The post and TM test had a significantly lower $FECO_2$, while the TM showed a higher FEO_2 compared to the pre-test ($p<0.05$, $p<0.01$, respectively). The TM test had a significantly lower magnitude of S_aO_2 decrease ($p<0.05$). MAP was significantly lower after apnea during the TM ($p=0.018$). $\dot{V}O_2max$ and the increase in apneic duration were moderately correlated ($r=0.505$, $p=0.027$). Lean mass and FVC were not significantly correlated to increases in EGP ($r=0.344$, $r=0.371$, respectively). **CONCLUSION:** Two weeks of daily CO_2 breath-hold table training appears to enhance apneic duration as well as the O_2 conserving effect of the MDR. Furthermore, using the CO_2 tables could shorten the vascular response recovery time. $\dot{V}O_2max$, FVC, and body composition do not appear to affect the physiological adaptations to increasing apneic duration.

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LIST OF ABBREVIATIONS

CO_2	Carbon Dioxide
MDR	Mammalian Dive Reflex
FVC	Forced Vital Capacity
$\dot{V}O_2max$	Maximum Oxygen Consumption
S_aO_2	Arterial Oxygen Saturation
HR	Heart Rate
MAP	Mean Arterial Pressure
$FECO_2$	Fraction of Expired Carbon Dioxide
FEO_2	Fraction of Expired Oxygen
Hb	Hemoglobin
TM	Timed Mimicked Test

CHAPTER ONE: INTRODUCTION

Free Diving

Documented human breath-hold diving began more than 2000 years ago in Japan, Korea, Greece, Persia and India, with the focus on gathering various sea creatures that were used as seafood or sold for profit.[1,2] A group still well known today in Japan and Korea, the Ama, perform dozens of dives per day to harvest seafood.[2] This simple method of food gathering has evolved into a multidimensional activity in modern culture.

Today, breath-hold (apnea) diving, also known as freediving, can be organized into two disciplines, competition and recreational. Freediving as a competition includes eight different events recognized by the International Association for Development of Apnea (AIDA).[3] The events consist of predominantly dynamic apneas but also include one static apnea event. Dynamic apnea can be defined as the requirement to produce motion using the body musculature during a breath-hold. Static apnea consists of having the body remain stationary and/or the absence of movement.[3] Each event has different dive performance goals and include diving for depth, distance, or time. There are also different devices/aids used during the competitions such as fins, weighted sleds, ropes, and inflatable bags to help the diver either descend or ascend in the water.[3] Currently, the male “No Limits Apnea” (NLT) world record is 214 meters (702 feet) set by Herbert Nitsch.[4] This event consists of riding a weighted sled to the prescribed depth and then inflating a bag with compressed air to return to the surface. Natalia Molchanova holds the female Static Apnea (STA) world record at 9 minutes (min) 2 seconds (s).[4] This event requires the athlete to remain submerged in a body of water during apnea. These are just a couple of events that are popular in competitive diving.

Recreational divers share technique and safety procedures with the competitive divers, but the goals and frequencies of each dive are different. The recreational side consists of spear fishermen, sea harvesters whose goal is not different from divers 2000 years ago, photographers, and site seers.[2] The depths descended to are not as substantial as those of competitive divers, but the number of dives can be impressive.[2,5] The Ama are known for completing approximately 100 dives throughout the day.[2] Furthermore, these divers are in search of sea creatures and pleasure rather than a new personal best or world record. This effectively places the frequency of dives as more important than depth, distance, or time.[6] These types of free divers can be described as repetitive free divers.[7]

Free diving at the competitive and recreational levels does not come without risk. Free divers push themselves to their limits in order to set new record depths or in attempt to spear an elusive fish. Unfortunately, sometimes the danger of the sport is all too real for the free diving community. The Divers Alert Network reported more than 30 deaths annually between 2006 and 2011.[8] A majority of these deaths were attributed to loss of consciousness from hypoxia during ascent.[9,10] Furthermore, loss of consciousness among amateur divers due to excessive hyperventilation is common.[9,10] Despite this, freediving continues to grow as new novice divers participate in the sport every year around the world.[1]

Learning to free dive has never been easier with the number of courses available from various organizations and for different levels of experience and skill.[3,11,12] A diver completing a beginner freediving course is expected to understand basic freediving techniques, dangers and risk, and safety procedures. These classes are important to provide a foundation to build upon for safe, enjoyable, and successful free diving.

As free divers become more advanced and comfortable in the water, the depth and duration of dives becomes more important. To do this, it is widely popular in the freediving community to implement the use of breath-hold tables for training. The tables prescribe breath-hold and breathing durations that are completed in a static, dry environment for safety precautions and convenience.[13] There are two different types of tables. Oxygen (O_2) tables provide a constant recovery time, while gradually increasing apnea duration. Conversely, carbon dioxide (CO_2) tables prescribe a constant apnea duration, while the recovery time gradually decreases.[13] O_2 tables are designed to adapt the body to hypoxic conditions, while CO_2 creates hypercapnic resiliency. These effects are beneficial to increase apneic duration.[1,5]

This study investigated three separate aspects of freediving: apneic duration, the dive response, and recovery. Apnea duration is the amount of time the diver is holding his/her breath after one inhalation to the time of exhale.[14] A longer apneic time correlates to an increased depth or opportunity to search and see. MDR is a complex, cardiovascular and respiratory reaction to apnea and is augmented with facial immersion. The end response is bradycardia, associated with peripheral vasoconstriction and a decreased cardiac output ($\dot{Q}$).[5,14] Lastly, specifically important to repetitive free divers, is dive recovery. This is the amount of time it will take for heart rate (HR), blood pressure (BP), and arterial blood O_2 saturation (S_aO_2), to return to resting levels post apnea. If repetitive divers can recover from each dive faster, they can repeatedly dive more and this correlates to increased sub-surface time, greater enjoyment, and greater hunting success.

Increasing the effectiveness of each component may result in new records, increased harvesting, more fun, and most importantly, safer diving.

Aspects of Free Diving

The Mammalian Dive Reflex (MDR) and its associated responses is essential for prolonging both static and dynamic apnea duration. It has been well documented as a bradycardic response with associated peripheral vasoconstriction due to immediate increases in parasympathetic and sympathetic activity.[5,14] The decreased HR and blood flow dramatically lower $\dot{Q}$.[15–17] The end result is a decreased O_2 uptake from the lungs, exhibiting an O_2 conserving effect.[17,18] This allows for prolonged apnea durations. However, the dive response and apnea duration are not the only characteristics that are important to free diving.

Dive recovery is an important consideration in regards to repetitive diving. Schagatay (2014) noted that the Ama require a short dive recovery to increase the overall time under the surface.[7] Schagatay (2005) explained a mechanism for this in an earlier study in which there was a decreased recovery time among trained free divers due to splenic contraction increasing hemoglobin (Hb) content in the blood.[19]

The effects of physical characteristics of free divers have been considered. Body composition has been considered to affect apneic time because of the thermoregulatory enhancements associated with an increased fat percentage; thus, maintaining a lower metabolic rate.[20] While lean mass has been noted to improve total blood volume and thus contributing to apnea duration.[20,21] Maximum oxygen consumption ($\dot{V}O_2$max) should be noted as an important factor to a diver due to the cardiovascular differences associated with an increased $\dot{V}O_2max$, such as higher hemoglobin concentration allowing for increased O_2 storage and CO_2 buffering efficiency.[22] Furthermore, research has shown positive correlations between $\dot{V}O_2max$ and total apneic duration.[23] Thus, $\dot{V}O_2max$ and its effects on apnea duration should be investigated further. Lastly, lung capacity is a crucial determinant of the amount of O_2 a free diver has available to him or her.[24] These physiological attributes may influence the overall dive performance of the individual.

The current research in apnea training is limited to 3 studies that use untrained individuals. Furthermore, the research neglects asking important questions from a multifaceted view. That is, how do physiological attributes and popular training methods affect free dive training? Engan (2013) implemented a 2-week apnea training program with untrained participants. The apnea training program consisted of 10 maximum breath-holds per day for 12 consecutive days.[22] After the apnea training intervention, the magnitude of HR reduction was significantly greater (69 ± 16 bpm vs. 61 ± 16 bpm, $p \leq 0.01$). Thus, exhibiting an accentuated dive reflex.[22]

Schagatay (2000) found similar results after untrained participants completed two weeks of 5 max-effort breath-holds per day with 2-min rest in between. The post-test revealed increases in HR reduction and apneic duration.[23] Both of these studies did not consider the effects physical attributes had on apnea training. They also included apnea protocols that are very different from breath-hold tables and training protocols currently used in the free diving community. Furthermore, there was an absence of recovery metrics (BP, HR, $S_aO_{2,}$) that would provide valuable information to repetitive divers.

The only other apnea training study known to date using untrained individuals investigated the effects of dynamic apnea training by triathletes. There was a 3-month intervention that had participants cycle at 30% of their $\dot{V}O_2max$ for 1 hour. During this bout of exercise, participants held their breath for 20 s intervals with 40 s of rest in between, totaling 60 apneas. Similar results were found to Engan and Schagatay: there was an accentuated dive reflex and static apnea duration post training. Additionally, blood acidosis was measured using venous blood pH and blood lactic acid concentration ([LA]). After training there was a significant decrease in both venous pH, and [LA].[25] This signifies that dynamic training could effectively decrease the recovery time between dives due to a decrease in blood acidosis.

Need for The Current Study:

Overall, the limited literature in apnea training has shown positive effects on improving the dive response and apneic time. However, there is a disconnect between the training protocols used in research and how the dive community actually trains. Furthermore, the literature has often neglected the effects of physiological attributes on apnea training. As more novice divers come to the sport every year, this study could provide valuable information to instructors and students. Lastly, the aspects of dive recovery have only been investigated in one of the three training studies published, which used triathletes as participants. This sample does not accurately represent the population of novice free divers, as these athletes $\dot{V}O_2max$ and other physiological variables would demonstrate significant training adaptations. This suggests that a more multifaceted static apnea training study focusing on untrained individuals would be beneficial to the free dive community.

Purpose

The purpose of this study is to determine if CO_2 breath-hold training tables are an effective means of enhancing the dive response, apneic time, and recovery in untrained individuals, as well as how physiological attributes affect differences in apnea training improvements.

Hypothesis

This study has five related hypotheses (H):

H1: After the completion of apnea training, it is expected that participants will demonstrate

- H1a: an increased apnea duration,
- H1b: accentuated MDR, and
- H1c: shorter recovery.

H2: Participants with a higher $\dot{V}O_2max$ will see greater improvements in the dive response, apneic time, and recovery post training.

H3: Participants with higher lean mass percentage will show more of an improvement post apnea training.

H4: Participants with greater lung capacities will have greater increases in apneic time and a reduced recovery time post training.

H5: Lastly, CO_2 breath-hold tables will prolong the easy-going phase of apnea.

Operational Definitions

Mammalian dive reflex (MDR): a cardiovascular and respiratory reaction to apnea with associated bradycardia, peripheral vasoconstriction, and decreased $\dot{Q}$.[14]

Untrained individuals are people who have not had any previous free diving or apnea training experience.

Recovery: This is the amount of duration it will take for HR, BP, and S_aO_2 to return to baseline levels post apnea.

Apnea duration is the amount of time spent from the inhalation of one breath until the time of exhalation.[14]

Delimitations/Limitations

The current study investigated how static apnea during cold face submersion is affected by dry static training. It did not simulate increased barometric pressure during training or testing. The use of increased barometric pressure would create unwanted variation between participants and measurements.[5] Furthermore, a dynamic apnea training intervention was not implemented. To better represent untrained free divers, static apnea was used as an intervention. During the 2-weeks of data collection, participants were asked to complete the apnea training independently. However, to increase the odds of compliance, participants met with the researcher 6 days into the intervention to monitor apnea training. It is also important to consider that diet could be an influencing factor during data collection.[20] Participants were asked to avoid food intake 2 hours before training each day to promote consistency of the metabolic response during apnea. However, the consumption of carbohydrates will differ between participants, thus could possibly affect the metabolic responses during the training period.[26]

Significance of Study

The completion of this study provides insight into apnea training that will benefit the free diving community. First, the study determined if the commonly used breath hold tables implemented in a 2-week static apnea training program enhanced the dive response, apneic time, and recovery among untrained individuals. Second, the study described how physiological attributes impact the effectiveness of apnea training.

CHAPTER TWO: EFFECTS OF TWO-WEEKS OF CO_2 BREATH-HOLD TABLE TRIANING ON THE DIVE RESPONSE AND APNEIC DURATION IN UNTRAINED DIVERS

Introduction

The mammalian dive reflex (MDR) and its associated responses is essential for prolonging both static and dynamic apnea duration. It has been well documented as a bradycardic response with associated peripheral vasoconstriction due to immediate increases in parasympathetic and sympathetic activity.[5,14] The decreased heart rate (HR) and blood flow dramatically lower cardiac output ($\dot{Q}$).[5] The end result is a decreased O_2 uptake from the lungs, exhibiting an O_2 conserving effect.[5,27] Maximal apneic attempts, composed of an easy-going phase (EGP) and struggle phase are separated by the physiological breaking point (PBP).[2] Defined as the point at which a critical level of partial pressure of carbon dioxide (PCO_2) stimulates central chemoreceptors, creating involuntary diaphragmatic contractions that precede the volitional breaking point of apnea.[28]

Surprisingly, few apnea training studies have been completed to investigate the adaptations that increase apneic duration and the dive response. Engan (2013) and Schagatay (2000) both implemented 2-week apnea training programs that consisted of 10 and 5 maximum breath-holds per day for 12 consecutive days, respectively.[22,23] After training, both studies found a prolonged easy-going phase. This was associated with an earlier onset and accentuated bradycardia, as well as probable increases in total vascular resistance.[22,23] Engan (2013) observed a decreased reduction in S_aO_2 after training during a series of breath-holds that matched the duration of the pre-test series known as timed mimicked (TM). This suggests a more efficient O_2 conserving effect after apnea training. The enhanced dive response, as well as a probable attenuated chemoreceptor sensitivity, is responsible for the increases in the EGP that was observed in both studies.[22,23]

In recent years, the use of what are known as “CO_2 breath-hold tables” has been growing in popularity, especially among new divers. The CO_2 tables consist of multiple submaximal dry apneas at a fixed duration.[29,30] After each apnea, the allowed recovery duration progressively decreases.[29–31] This is thought to gradually increase PCO_2, therefore increasing hypercapnic resiliency.[29–31] To date, a training study implementing the currently applicable CO_2 breath-hold tables has not been performed. Therefore, the purpose of the current study is to investigate whether or not CO_2 training tables are appropriate means of increasing apneic time, and if so, what mechanisms are responsible for these enhancements. We hypothesized that apneic duration, from an increased EGP, will be increased due to an accentuated dive response after training.

Methods

Participants

Nineteen healthy individuals with no previous experience in breath-hold training were recruited to complete a 12-day apnea training program. Four participants did not complete the breath-hold training program; thus, the data of 15 participants (9 males, 6 females, Table 1.) were used for analysis. All procedures were approved by the Institutional Review Board and participants provided consent and completed the PAR-Q.[32]

Table 1. Apnea training study participant characteristics

Characteristic	*Mean*	*Std. Deviation*
Age (years)	22.67	4.85
Height (m)	1.74	0.11
Weight (kg)	72.37	13.2

Apnea Training Program

Based on the CO_2 apnea training protocols commonly used among novice free divers, the 15 participants completed 8 sub-maximal apneas per day for 12 days. The 8 apneas had a constant duration with a gradually decreasing recovery time (Table 2). Both the duration of apnea and recovery were prescribed from the maximum-effort dry breath-hold duration. Participants were instructed to lie in a supine position, to not hyperventilate, and to perform maximal inhalations prior to completing the breath-hold table. Successful completion of each training session was recorded in an online log electronically shared with the investigator.

Table 2. CO_2 Group: Breath-Hold and Recovery Duration (% Dry Apnea

Apnea #	Breath-Hold Duration (% Dry Apnea)	Recovery Duration (% Dry Apnea)	Successful Completion of Breath-Hold (Yes or No)
1	50%	80%	
2	50%	75%	
3	50%	70%	
4	50%	65%	
5	50%	60%	
6	50%	55%	
7	50%	45%	
8	50%	Recovery	

Procedures

Participants were advised not to eat heavy meals or perform moderate to vigorous exercise before data collection. Height and weight were collected in the first session, body mass (BMB-800S scale, Tanita corporation of America, Arlington Heights, IL) and height (stadiometer, Seca, Chino, CA). Other physical characteristic testing can be found in the Appendix A as they were not applicable to the current manuscript. Participants were also familiarized with the facial immersion (FI) testing to occur during the next data collection. See Figure 1. for the study outline.

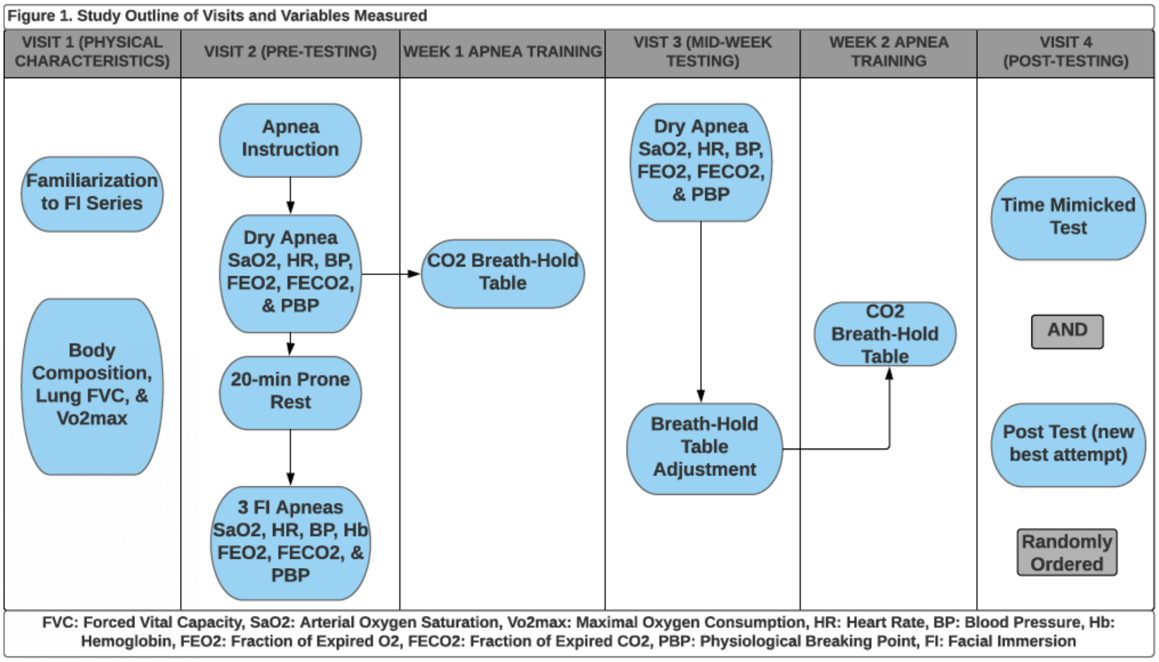

Figure 1 Study outline of visits and variables measured.

Baseline Testing

Within 2 weeks of the initial data collection, baseline testing was performed. Two different conditions consisting of dry and facial immersed (FI) apnea were measured at both baseline and after apnea training. Throughout both conditions S_aO_2, HR, and respiratory movements were continuously recorded using a finger pulse oximeter, plethysmograph, and respiratory transducer (MP36 Data Acquisition Unit, BIOPAC Systems, Goleta, CA). Blood pressure was measured with an automatic wrist blood pressure cuff (HoMedics, Commerce Township, MI) before, during, and after breath-holds.

Prior to the dry apnea, participants were asked to lie supine on a cushioned gurney for 20 minutes for resting data collection. At 10 minutes before apnea, the participants inserted the gas collection mouthpiece for measurement of oxygen consumption ($\dot{V}O_2$) using the TrueMax 2400 (ParvoMedics, Salt Lake City, UT) metabolic cart. Participants were given a warning at 2 minutes, 1 minute, and 30 seconds before starting apnea. At 10 seconds, a countdown began followed by the participant's maximal inspiration and a maximal duration breath-hold. Blood pressure was measured at 30 seconds into the apnea while HR, respiratory movements, and S_aO_2, were measured continuously.

At the cessation of apnea, participants remained in the supine position for 4 minutes while recovery data were obtained. This included fraction of expired oxygen (FEO_2) and fraction of expired carbon dioxide ($FECO_2$). Blood pressure was measured at 30 seconds, 1 minute, 2 minutes, and 3 minutes after apnea.

Next, participants were asked to perform 3 prone FI apneas. Prior to and in between FI, participants rested their head on a pillow that covered the cold-water tub (6-8℃). At 5 seconds before apnea participants were asked to lift their head, the pillow and lid were removed, and at 1 second participants were instructed to take a maximal inhalation, set their face from forehead to chin into the water and then hold their breath as long as possible. At the cessation of apnea, the cover and pillow were replaced and face dried. This was repeated two more times with 2 minutes between each trial. At the end of the third and final FI apnea 4 minutes of recovery data were collected.

Mid and Post Testing

After 4 to 6 days of training, participants completed mid-testing which followed the same protocol as the dry apnea pre-test. The mid-test apnea was used to prescribe a new CO_2 training table utilizing the same percentages. After the completion of all 12 days of training, the participants completed 3 FI apneas for post-training testing and time-mimicked-testing (TM), in random order, separated by 20 minutes of rest. Before both conditions, resting HR and BP values were obtained. During the FI TM testing participants held their breath for same duration and order as each of their pre-test FI apneas.

Data Analysis

All participants served as their own controls. Pre and post-tests were compared to examine the differences of max effort apneic durations. The TM and pre-tests were compared to examine the physiological adaptations over the same apneic duration.

The physiological breaking point (PBP) was used to differentiate between the "easy-going phase" and associated secondary "struggle phase" during apnea. The PBP can be defined by the first involuntary diaphragmatic contraction during apnea. This is a result of reaching a P_aCO_2 threshold that stimulates inhalation during apnea.[28]

HR mean was taken 10 seconds before each apnea. This was used as a baseline for both HR onset, as well as magnitude of HR decrease. The time from the start of apnea to when HR dropped below one standard deviation from baseline was defined as the HR onset time. The lowest HR achieved during the breath-hold was measured as a 10 second mean. This mean was used to calculate magnitude of drop from the baseline HR.

A 10 second baseline S_aO_2 mean was obtained immediately before each apnea. The magnitude of S_aO_2 drop was determined from using the baseline value as well as the nadir S_aO_2.

Statistics

All analyses were performed with IBM SPSS Statistics 25 (IBM Corporation, Armonk, NY). To determine if the dive response or apneic duration was enhanced, paired t-test and repeated measures ANOVA were used to compare the pre, post, and TM values: EGP duration, HR, BP, S_aO_2, $FECO_2$, and FEO_2. Data are presented in means ± SD with an alpha level ≤ 0.05.

Results

Apneic Time

The post FI3 apneic duration was significantly longer than the pre ($p<0.01$) as was the easy-going phase in both the post and TM FI3 apnea ($p=0.008$ and $p=0.009$, respectively). In regards to the struggle phase, it was decreased in the TM FI3 ($p=0.022$) and increased in the post-test ($p<0.05$). (Table 3.)

Table 3. Duration of Apnea Phases and Conditions

Phase/Condition	Pre-FI3	Post-FI3	TM-FI3
Apneic time (s)	67.12 ± 25.24	96.88 ± 44.97**	68 ± 25.09
Easy-going time (s)	43.14 ± 15.15	58.74 ± 19.06**	58.11 ± 25.71**
Struggle time (s)	27.83 ± 13.46	43.55 ± 26.25*	15.39 ± 10.36

*= Significant difference from Pre-FI3 ($p<0.05$), **=Significant difference from Pre-FI3 ($p<0.01$)

Cardiovascular Response

Heart Rate

There were no significant differences between the pre, post, and TM baseline HR values ($p>0.05$). All tests showed a significant decrease from their respective baseline HR values ($p<0.001$). There were no significant differences between HR minimum averages before and after apnea training. The rate of onset during the post and TM apneas did not differ from the pre-test ($p>0.05$).

Mean Arterial Pressure

The resting MAP values were not significantly different between the pre, post, and TM test ($p>0.05$). The MAP during apnea was significantly greater than the resting MAP for pre, post and TM ($p=0.033$, 0.001, and 0.009, respectively). The magnitude of MAP increase was higher during both the post and TM test compared to the pre-test, however, not significant ($p=0.93$, $p=0.49$, respectively).

Arterial Oxygen Saturation

The pre, post, and TM test showed no statistical differences in resting S_aO_2 ($p>0.05$). Although there was a higher magnitude of S_aO_2 decrease after training, it was not significant ($p=0.42$). The TM-test had a significantly lower magnitude decrease in S_aO_2 compared to the pre-test (2.78 ± 0.64% vs. 1.46 ± 0.43%, $p=0.017$, respectively).

Gas Exchange

The resting $\dot{V}O_2$ values obtained before the start of each apnea series were not significantly different ($p>0.05$). The $FECO_2$ significantly decreased during the post-test (4.96 ± 0.61 vs. 4.75 ± 0.55, $p=0.021$) and the TM test (4.88 ± 0.64% vs. 4.65 ± 0.56%, $p=0.005$). There was a decrease in FEO_2 after training during the post-test, however, this was not significant ($p=0.072$). The TM FEO_2 proved to be significantly higher than the pre-test (14.79 ± 0.85% vs. 14.33 ± 1.16%, $p=0.006$, respectively). The FEO_2 during the TM-test was also significantly higher than the post-test (14.79 ± 0.85% vs. 14.06 ± 1.09%, $p<0.001$, respectively). See Figures 2. and 3.

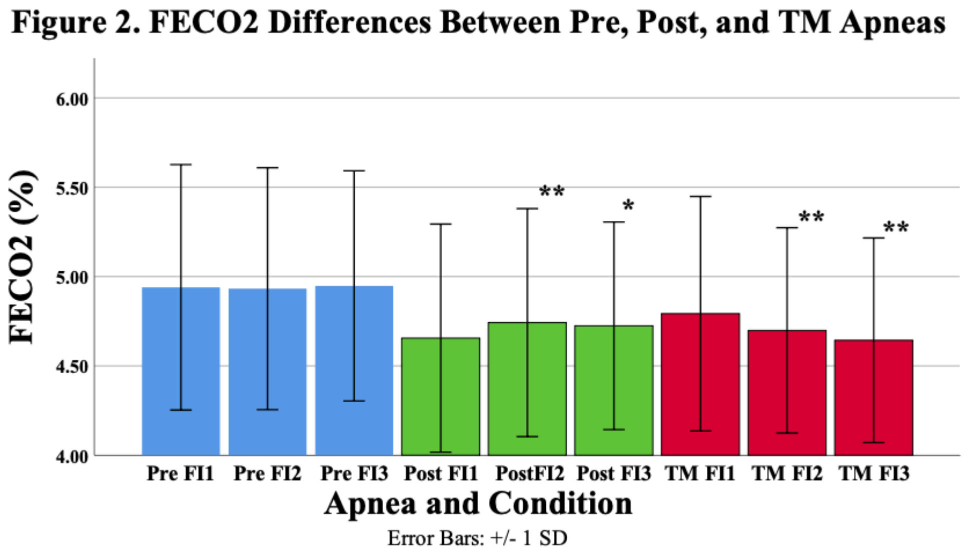

Figure 2. **FECO2 Differences Between Pre, Post, and TM Apneas**

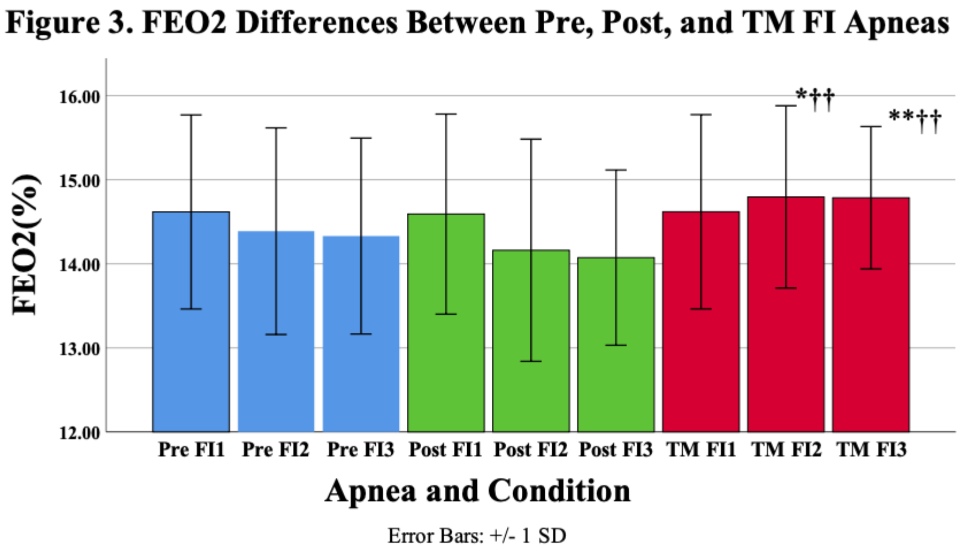

Figure 3. **FEO2 Difference Between Pre, Post, and TM FI Apneas**

*= Significant difference from the respective pre-apnea ($p<0.05$), **=Significant difference from respective pre-apnea ($p<0.01$), ††= Significant difference from respective post-apnea ($p<0.01$).

Discussion

This study found a prolonged apneic duration as well as an increased EGP after apnea training. This was associated with a lower magnitude decrease of S_aO_2 and FEO_2

during the TM test. There was also a decreased $FECO_2$ during the post and TM test. There were no significant differences for the bradycardic and vascular responses after training.

A main finding of this study was an increase in total apneic duration during the post-test. This was due to significant increases in both the EGP and the struggle phase. The TM-test revealed similar results with an increased EGP. The end of the EGP, known as the PBP, is determined by the first involuntary diaphragmatic contraction during apnea, and is brought on by surpassing a critical threshold of CO_2 accumulation.[33] Thus, the improvements of apnea duration in this study can be attributed to a physiological rather than a psychological adaptation. A possible mechanism for the prolonged EGP is a decrease in central chemoreceptor stimulation. Furthermore, trained divers can tolerate a higher partial pressure of CO_2 as compared to non-divers.[1] A decreased CO_2 sensitivity has been suggested in human divers who are commonly exposed to high arterial PCO_2.[34,35] Furthermore, a blunted ventilatory CO_2 response has been observed in both diving mammals and the Ama, a group well known for completing dozens of breath-hold dives per day.[36,2] This study found a significant decrease in $FECO_2$ in both the post and TM tests, supporting the previous literature of a blunted ventilatory CO_2 response. This has also been suggested to be evident in experienced divers more so than in the untrained.[1] In addition, two previous apnea training studies that exhibited similar EGP duration responses suggested that either a decreased P_aCO_2 chemoreceptor sensitivity or CO_2 production could be responsible.[22,23]

Another possible mechanism responsible for prolonging the EGP is the enhancement of the metabolic restricting dive reflex.[22,23] The associated bradycardia and peripheral vasoconstriction response have been well documented to have an O_2 conserving effect.[20,37] This is probably due to decreased apneic lung-to-blood O_2 uptake.[18] This effect was evident in the current study by the attenuated decrease in S_aO_2, as well as the significantly higher FEO_2 during FI2 and FI3 TM apneas. Two previous apnea training studies by Engan (2013) and Schagatay (2000) found a more pronounced and earlier bradycardic response associated with a prolonged EGP after two weeks of daily maximal apneas.[22,23] One of these studies also found an attenuated S_aO_2 decrease during TM testing, supporting that the O_2 conserving effect is enhanced with a more efficient bradycardic response. However, the current study did not observe either an increased magnitude or earlier onset of bradycardia. A previous study compared O_2 delivery and the dive reflex between divers and non-divers.[38] In alignment with this study, the magnitude of HR reduction between the two group was not different across five facially immersed apneas.[38] The same five apnea immersions protocol was used by Schagatay (2000) who found a greater magnitude and earlier onset of bradycardia.[23] Furthermore, Engan (2013) used 3 apneas without the use of facial immersion.[22] As found by others, facial immersion attenuates the magnitude of bradycardia.[39,40] Thus, the differences in the apneas protocol could be responsible for the differences in results.

The vascular response is another mechanism that could explain the enhanced O_2 conserving effect. Increased vascular resistance from peripheral vasoconstriction decreases blood flow in the extremities, leading to a decreased muscle O_2 uptake. [41,42,43,44] Both previous apnea training studies suggested that the similar MAP with decreases in HR after apnea training were the result of an increase in vascular resistance.[22,23] The current study did not observe enhancements in the bradycardic response, nor differences in MAP after training. However, it should also be considered this study was limited to one measurement of MAP 30 seconds into apnea. It has been suggested that hypoxia strengthens vasoconstriction.[5,45] Therefore, it should be questioned if the full potential of the vascular response during this study was measured. In this study, apnea duration was far longer than 30 seconds and would inevitably increase the degree of hypoxia and therefore vasoconstriction. Thus, the possibility remains, that an enhanced O_2 conserving effect after apnea training could possibly be from an increase in vascular resistance.

The differences in apnea training protocols could be responsible for different findings among the cardiovascular responses. The CO_2 breath-hold tables that were implemented required the participant to complete 8 breath-holds at 50% maximal duration, followed by gradually decreasing recovery after each apnea. Engan (2013) implemented a two-week training program requiring two series of five maximal effort apneas with two minutes of rest between apneas. Each apnea was preceded by 1 minute of hyperventilation to decrease arterial carbon dioxide ($PaCO_2$).[22] Thus, it is assumed that the CO_2 breath-hold tables would produce a higher degree of hypercapnia and a lesser degree of hypoxia, while the opposite is true for the latter. These differences could have implications for the physiological adaptations in regards to the dive reflex as well as chemoreceptor stimulation. Hypoxia has been found to decrease the time of onset of vasoconstriction, while also augmenting the magnitude.[45,46] Thus, perhaps the present apnea training program did not adequately provide a hypoxic stimulus for peripheral augmentation of vasoconstriction. In contrast, the increased hypercapnia provided sufficient stimulus to decrease chemoreceptor sensitivity. This would align well with the purpose of the CO_2 tables that were implemented, which is increasing hypercapnia resiliency. In contrast, O_2 tables that provide a constant recovery time with gradually increasing apneic durations, are meant to increase hypoxic resiliency, which was not observed in the present study.

There are limitations that should be considered in the present study. The absence of continual blood pressure measurement could be a limiting factor in order to assess the full magnitude of the vascular response. Furthermore, the absence of cardiac output and stroke volume measurement made it difficult to assess the magnitude of the MDR. Also, this study did not measure gas exchange during the training intervention, which could have added validity to the assumption that CO_2 training tables increase hypercapnia disproportionally to hypoxia.

Future research should consider testing the validity of the commonly used CO_2 training tables. That is, does the gradual decreased recovery time increase intravenous and ventilatory PCO_2 as expected? There could also be interesting findings while measuring the dive reflex during training, which could provide insight into training specific responses, as well as to better rationalize the findings in the current study. Also, future research should consider training adaptations with the implementation of O_2 training tables, that utilize a constant recovery time with gradually increasing apneic durations. This research could perhaps give insight into different training methodologies and their effects on training adaptations.

In summary, two weeks of daily CO_2 breath-hold table training appear to supply an adequate stimulus for enhancing apneic duration as well as the O_2 conserving effect. However, more research is required to determine if an increased vascular response is responsible. It appears that the commonly used CO_2 tables in the free dive community are beneficial to novice free divers.

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APPENDIX A

Additional Methods

Participants

Nineteen healthy individuals with no previous experience in breath-hold training were recruited for the study. Four participants did not complete the breath-hold training program. Thus, the data of 15 participants (9 males, 6 females, Table 1.) were used for analysis. Participants were recruited by word of mouth, email, and paper announcements. During apnea, there are changes in metabolic rate associated with stresses on the cardiovascular and pulmonary systems due to hypercapnia and hypoxia.[5,14] For these reasons, the exclusion criteria included: not within 18 to 40 years of age, smokers (or using products containing nicotine), people with metabolic, cardiovascular, or pulmonary diseases, or medication that affects metabolic rate. Before data collection, participants received and signed an informed consent as well as a Par-Q.

Instruments and Measures

Body Composition

Body mass was measured in kilograms with a BMB-800S scale (Tanita Corporation of America, Arlington Heights, IL). A stadiometer (Seca, Chino, CA), was used to measure height to the nearest tenth of a centimeter. Percent body-fat (%BF) and lean mass (%LM) was estimated with the use of a BOD POD (Cosmed, Rome, Italy). This method of body composition testing has shown to be valid and reliable.[47,48]

Forced Vital Capacity (FVC)

The FVC of participants was measured with the use of a Pony FX Desktop Spirometer (Cosmed, Rome, Italy).

Blood Hemoglobin Content (Hb)

A G3000-CONTCUV HemoPoint H2 Hemoglobin Analyzer Photometer (EKF Diagnostics-Standbio Laboratory, Elkhart, IN) was used to measure Hb. Blood was sampled from a finger stick.

$\dot{V}O_2max$

A TrueMax 2400 metabolic cart (ParvoMedics, Salt Lake City, UT) and Woodway treadmill (Woodway, USA, Inc, Waukesha, WI) were used to measure maximum oxygen uptake ($\dot{V}O_2max$) with a Bruce protocol implemented.[33]

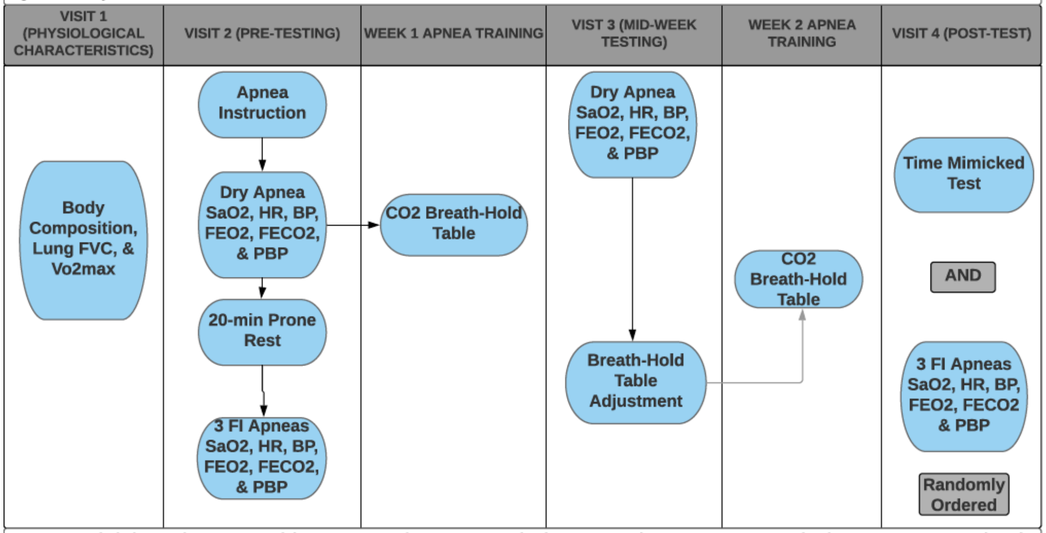
Procedures

Visit One: Overview/Informed Consent/Physiological Data Collection (approximately 1.5hr)

The participants met with the investigator in the Human Performance Lab (HPL) for a total of 4 visits. During the first visit, the general purpose and protocol were explained and any questions answered. If the participants agreed to take part in the study, they were asked to sign an IRB approved consent form.

Anthropometric data including height and weight was measured and BMI (kg/m^2) calculated. Following the manufacturer's protocol, the BOD POD, spirometer, and metabolic cart were calibrated before each initial visit. Percent body fat and lean mass were estimated using a BOD POD. After this, forced vital capacity was measured with a spirometer. The participants then completed a maximal oxygen consumption test using the Bruce protocol. The results from testing were withheld from the participants until they completed the study. At the conclusion of testing, the investigator and participants scheduled the next 3 visits. The investigator also asked the participants to refrain from the following before the next visit: vigorous exercise 12 hours before, food or liquid consumption 1 hour before, heavy meals or products containing caffeine 2 hours before. Approximately 24 hours before the second visit, an email was sent reminding participants of the appointment time and place as well as the items to be avoided.

Figure 1. Study Outline of Visits and Variables Measured



FVC-Forced Vital Capacity, SaO2-Arterial Oxygen Saturation, Vo2max-Maximal Oxygen Uptake, HR-Heart Rate, BP-Blood Pressure, FEO2: Fraction of Expired O2, FECO2: Fraction of Expired CO2, PBP- Physiological Breaking Point

Data Analysis

The time it took for HR, S_aO_2, and $\dot{V}O_2$ to reach resting values after the FI apnea series was recorded in seconds.

Statistics

All analyses were performed with IBM SPSS Statistics 25. To determine if there was a relationship between physical characteristics and apnea performance, correlation analyses were performed using FVC, %BF, %LM, and $\dot{V}O_2max$ as the independents, and the following as the dependent variables: total apneic duration, EGP and struggle phase duration, HR, BP, S_aO_2, and $FECO_2$ and FEO_2.

APPENDIX B

Additional Results

The following data were collected during the study. However, this section, as well as the Additional Discussion were left out of the manuscript in order to focus on the effects of apnea training separate to recovery and physical characteristics.

Recovery

Mean Arterial Pressure

The MAP pressure measured 30 seconds after FI3 was similar between pre and post apneas (96.75 ± 9.17 vs. 95.14 ± 10.69 mmHg, $p=0.550$, respectively). The TM FI3 had a significantly lower MAP (92.32 ± 7.33 mmHg, $p=0.018$) 30 seconds after apnea compared to the pre-test. The pre-test MAP after apnea was significantly higher when compared to its respective resting value (96.75 ± 9.17 vs. 90.88 ± 7.82 mmHg, $p=0.019$). MAP after apnea for post and TM tests were not significantly different from their respective resting values ($p>0.05$). MAP at one, two and three minutes after apnea revealed no significant differences after training.

Heart Rate

All participants reached resting HR values within 5 seconds after the cessation of apnea. There were no differences after apnea training.

Oxygen Consumption

After apnea training, there were no significant differences between pre and post $\dot{V}O_2$ recovery durations (43 ± 22 sec vs. 42 ± 20 sec, $p=0.9$, respectively. The TM recovery time (33 ± 10 sec) was shorter than pre-testing, however, not significant ($p=0.10$).

Arterial Oxygen Saturation

There were no significant differences between pre (45 ± 21 sec), post (37 ±10 sec), or TM S_aO_2 recovery durations (42 ± 20 sec, $p>0.05$ for all).

Physical Characteristics

Body Composition

Increases in FI3 apneic duration were inversely correlated to percent fat mass (r= -0.219, p=0.216). In contrast, percent lean mass was directly correlated to increases in apneic duration (r=0.219, p=0.216). Percent fat mass was inversely correlated to FI3 EGP duration from pre to post, while lean mass was directly correlated (r=-0.344, r=0.344, p=0.165, respectively). These findings were not significantly correlated.

Maximal Oxygen Consumption

$\dot{V}O_2max$ was directly correlated to the TM EGP (r=0.592, p=0.036), as well as total apneic duration for the pre, post and TM test (r=0.797, r=0.723, r=0.803, $p<0.001$, p=0.001, $p<0.001$, respectively). $\dot{V}O_2max$ was directly correlated to the struggle phase during the pre and post-test (r=0.724, r=0.714, p=0.009, p=0.01, respectively). A strong correlation was made between $\dot{V}O_2max$ and increases in FI3 apneic duration (r=0.505, p=0.027), while a direct correlation was made to increases in the EGP (r=0.45, p=0.096), however, not significant.

Forced Vital Capacity

FVC was strongly and directly correlated to the EGP after apnea training (r=0.667, p=0.016). There was a small correlation to FI3 apneic duration increase from pre to post (r=0.134, p=0.318), and a moderate correlation to increases in the EGP from pre to TM apnea (r=0.371, p=0.145). See Table 4. for physical characteristics of participants.

Table 4. Apnea training study participant physical characteristics

Characteristic	*Mean*	*Std. Deviation*
% Fat	16.27	7.09
% Lean	83.73	7.09
$\dot{V}O_2max$ *(ml/kg/min)*	53.72	12.55
FVC (liters)	5.23	1.33

Hematological

Due to insufficient blood draws via finger stick, the following data is from 9 participants (n=9). Resting hemoglobin (Hb) values were not significantly different across pre, post and TM tests (13.9 ± 1.0, 15.2 ± 2.0, 14.8 ± 0.8 g/dl, p>0.05, respectively). There were no significant differences between resting values and FI3 Hb for the pre (14.0 ± 1.1 g/dl), post (15.0 ± 1.1 g/dl) and TM (15.5 ± 1.4 g/dl) tests. All p>0.05.

APPENDIX C

Additional Discussion

Recovery

The dive response is beneficial during apneas due to its O_2 conserving effect, however, the opposite is true during recovery.[5] If vasoconstriction and bradycardia were to continue after apnea the result would be a decreased O_2 saturation and uptake by the body, as well as a decreased efficiency of expelling CO_2.[19] Therefore, it is crucial for the dive response to dissipate immediately after the cessation of apnea. The current literature is very limited regarding this topic. The MAP measured 30 seconds after apnea was similar between pre and post-tests. However, the TM apnea had a significantly lower MAP compared to the pre-test, suggesting that there was a training induced adaptation allowing for the more rapid recovery of the vascular response. Furthermore, the pre-test MAP 30 seconds after apnea was significantly higher than its resting value. In contrast, the TM and post-test MAP after apnea was not significantly higher than resting values. Therefore, a more efficient vascular recovery was evident after training. Perini (2010) found blood pressure after static maximal apneas to reach resting values within 30 seconds after apnea, which was evident in the post and TM tests, but not the pre-test.[49] Perini's study used expert breath-hold divers, suggesting that the ability for vasoconstriction to return to resting values could somewhat depend on apnea training or experience.[49]

The current study found that HR increased back to resting values within 5 seconds after the cessation of apnea and was not different after training. Similar findings were made by Perini (2010) who found HR to return to resting values within 5 seconds post apnea. Perini also found no change in stroke volume, resulting in $\dot{Q}$ returning to resting by 10 seconds.[49] The current study did not measure $\dot{Q}$, therefore a true comparison of cardiovascular recovery between the two studies is difficult.[49] However, it is clear the cardiac response ceases quickly after breathing resumes. Furthermore, the vascular response after apnea may be mediated by other factors rather than the cessation of the dive reflex. This could be the degree of hypoxia, which was blunted during the TM test shown by a higher S_aO_2. To test this, future research should investigate different levels of hypoxia in trained divers and compare it to MAP recovery. The faster MAP recovery did not improve the rate of $\dot{V}O_2$ recovery after training. Also, there was no difference in S_aO_2 recovery. A significantly longer apnea time during the post test, yielding similar O_2 recovery metrics could be demonstrating a training adaptation.

The active contraction of the spleen has also been suggested as a component of the dive response in recent years.[19,50] As the spleen decreases in volume, there are significant increases in the amount of hematocrit (Hct) and Hb.[51] The increased red blood cells (RBC) have obvious benefits for O_2 and CO_2 storage capacity.[20] The average human spleen stores about 200-250 ml of blood.[52] If the full amount is utilized from the splenic contraction it equates to about a 2-4% increase of RBC's.[53] In the Weddell seal it has been shown that spleen contraction is inversely related to catecholamine release.[54] Similarly in humans, the contraction is most likely initiated through increases in hypoxia which sympathetically stimulate the release of catecholamines triggering the smooth muscle around the spleen to contract.[55] Engan (2013) investigated hemodynamics before and after apnea training. Although the splenic response was evident during the pre and post-testing, there was no augmented response after two weeks of apnea training.[22] The current study did not observe the splenic response as there were similar Hb values before and after apneas for all three tests. It is possible the participants did not reach a sufficient hypoxic state to elicit significant increases in Hb. Prommer (2007) found no increases in Hb in the untrained after 5 apneic dives.[56] However, other studies found three repeated apneas to be sufficient for eliciting a splenic response.[19,22] The lack of Hb increase in the current study could also be the result of an insufficient technique to draw blood as missing data was evident.

Physical Characteristics

This is the first study to date to investigate how physical characteristics affect improvements associated with apnea training. The current study found muscle mass to be positively moderately correlated to increases in EGP duration; however, this was not significant with this number of participants. Increases in muscle mass provides the added benefit of increases in blood volume, an aspect that increases O_2 storage capacity,[20,21,57] but increases in muscle mass will also require a greater O_2 demand.[58] However, the vascular response effectively reduces O_2 uptake by the muscle, suggesting an efficient diving response could limit the latter.[14,46]Although the current study did not observe significant correlations between muscle mass and the EGP, it seems reasonable to suggest that greater muscle mass is more beneficial for improving static apnea duration rather than lesser muscle mass. As suggested by others, this is due to the increased blood volume.[20]

Marine mammals have a greater number of mitochondria, greater utilization of fat metabolism for energy, as well as increased myoglobin for gas storage to help resist hypercapnia and hypoxia.[59,60] These attributes allow marine mammals to sustain aerobic metabolism throughout apnea.[59,60] These same benefits have been found in humans with a higher $\dot{V}O_2max$.[61] The current study investigated whether or not these adaptations could be better utilized after apnea training. Post training, there was a strong positive correlation between $\dot{V}O_2max$ and total apneic duration for the pre, post and TM tests. Although the correlation to the EGP was found during the TM test, the struggle phase was strongly correlated to both the pre and post-testing. Schagatay (2000) found the struggle phase duration to increase after long-term physical training with increases in $\dot{V}O_2max$, while the EGP duration was unchanged.[23] These results suggest that aerobic capacity shares a stronger relationship to the struggle phase rather than the EGP. This study also found significant correlations between $\dot{V}O_2max$ and apneic improvement time. There was no significant correlation of EGP improvement to $\dot{V}O_2max$. These findings suggest that people with a higher $\dot{V}O_2max$ will have greater improvements in the struggle phase. Therefore, psychological rather than physiological resiliency is responsible for the correlations to $\dot{V}O_2max$.[23] Future research should investigate the effects of $\dot{V}O_2max$ on apnea during exercise. Also, further investigating the psychology of apnea training may yield beneficial material to the free dive community. That is, which apnea training interventions yield the greatest improvements in psychological resiliency.

Previous studies have found that higher lung capacities contribute to longer apnea durations.[62] The same is true for the current study as the EGP after apnea training was strongly correlated to FVC. However, the current study investigated whether or not larger lung volumes would show increased improvements in apnea duration after training. There were no significant correlations made between FVC and total apneic or EGP duration. These results suggest that people with smaller lung volumes would benefit from apnea training proportionally to people with larger lung volumes. However, one should consider the use of CO_2 tables while interpreting these results. That is, rather than training to withstand a hypoxic stimulus, CO_2 tables are meant to stimulate hypercapnic resiliency.[29–31] Furthermore, the present CO_2 tables prescribed apnea durations at 50% of maximal dry attempts. Thus, the full O_2 storage potential of different lung volumes are not being utilized in the current apnea training program. One should consider investigating training programs that utilize maximal apneic attempts, or the commonly used O_2 breath-hold table.

The current study concludes that two-weeks of CO_2 table breath-hold training could shorten the vascular recovery time after the cessation of maximal effort apnea. Also, differences in $\dot{V}O_2max$ and FVC do not appear to affect physiological adaptations in regards to EGP duration. These findings imply that people with a higher $\dot{V}O_2max$ will be able to withstand longer struggle phases of apnea, thus, psychological resiliency adaptations are most likely responsible. Therefore, free divers should consider increasing their $\dot{V}O_2max$ as a means of psychological training for apnea. Future research should consider this in participation recruitment in apnea studies, as well as investigate further if there are any physiological adaptions that could be responsible for relationship of $\dot{V}O_2max$ and an augmented struggle phase.