CEREBROVASCULAR REACTIVITY OF FREE DIVERS MEASURED WITH fNIRS

by

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ABSTRACT

CEREBROVASCULAR REACTIVITY OF FREE DIVERS MEASURED WITH fNIRS

Although breath-hold diving is nowadays practiced as a competitive sports discipline, its underlying physiological mechanisms enabling divers to tolerate great dive depths and durations are still not fully understood. Effect of consecutive maximal dry breath-holds was compared between two groups of free divers with different experience levels and a control group. Hemoglobin concentration (by functional near infrared spectroscopy, fNIRS), heart rate and systemic oxygen saturation (by pulse oxymetry) measurements were performed during four consecutive maximal dry breath-holds. Breath-hold durations increased with consecutive trials in all the groups while the experienced free divers outperformed both the beginner free divers and the control group. Δ[Hb] from fNIRS measurements increased in parallel with increasing breath-hold durations in free divers but remained almost constant in the control group. SO_2 decreased with increasing breath-hold durations for all the groups with a greater decrease for experienced free divers due to longer breath-hold durations. Breath-hold indices calculated using Δ[Hb] and independently using ΔSO_2 normalized to hold durations showed significant differences between groups ($p<5 \times 10^{-5}$ for both indices). Free diver groups exhibited a higher slope of increase of the indices among consecutive breath holds compared to the control group elucidating an enhanced reactivity to hypoxia. Our results indicate that cerebrovascular reactivity to hypercapnia can be learned and trained and the level of reactivity can be reliably quantified by fNIRS.

Keywords: functional Near Infrared Spectroscopy, Apnea, Breath-hold, Divers.

ÖZET

SERBEST DALGIÇLARDA iYKAS İLE SEREBROVASKÜLER REAKTİVİTE ÖLÇÜMÜ

Serbest dalış günümüzde yaygınlaşmış ve müsabakaları olan bir spor dalı haline gelmiş olsa da, dalgıçların muazzam derinliklere inebilmelerinin ve uzun süre su altında kalabilmelerinin temelinde yatan fizyolojik mekanizma henüz tam olarak anlaşılamamıştır. Ardışık maksimal nefes tutmaların etkileri, farklı deneyim düzeylerine sahip iki serbest dalgıç grubu ve kontrol grubu üzerinde incelenmiştir. Dört ardışık maksimal nefes tutma boyunca, hemoglobin konsantrasyonu (işlevsel yakın kızıl altı spektroskopi (iYKAS) ile), kalp atış hızı ve sistemik oksijen satürasyonu (nabız oksimetrisi ile) ölçümleri alınmıştır. Nefes tutma süreleri bütün gruplarda birbirini takip eden denemelerde uzamıştır. Ancak deneyimli serbest dalgıçlardaki uzama, yeni başlayan dalgıçlara ve kontrol grubuna göre daha uzundur. iYKAS ölçümlerinden hesaplanan $\Delta[\text{Hb}]$ değerleri, serbest dalgıçlarda uzayan nefes tutma sürelerine paralel olarak artış gösterirken, kontrol grubunda neredeyse sabit kalmıştır. SO_2 değerleri ise bütün gruplar için uzayan nefes tutma süreleriyle birlikte bir azalma göstermiştir. Deneyimli serbest dalgıçlardaki azalma ise daha uzun nefes tutma sürelerine bağlı olarak daha fazladır. $\Delta[\text{Hb}]$ değerleri ve bağımsız olarak ΔSO_2 değerleri kullanılarak hesaplanan ve nefes tutma süreleri ile normalize edilmiş nefes tutma indeksleri gruplar arasında istatiksel olarak farklıdır ($p < 5 \times 10^{-5}$ her iki indeks için). Serbest dalgıç grubunun ardışık nefes tutma indekslerindeki artış, kontrol grubunun indeks artışına göre daha fazladır ve bu da hipoksiye karşı daha gelişmiş bir tepki verdiklerini gösterir. Sonuçlarımız hiperkapniaya karşı verilen serebrovasküler tepkinin öğrenilebildiğini ve eğitilebildiğini ve tepki düzeyinin iYKAS ile güvenilir bir biçimde ölçülebildiğini göstermektedir.

Anahtar Sözcükler: işlevsel Yakın Kızıl Altı Spektroskopi, Nefes tutma, Serbest dalış.

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LIST OF ABBREVIATIONS

O_2	Oxygen
CO_2	Carbon-dioxide
[Hb]	Hemoglobin concentration
SO_2	Systemic oxygen saturation
Δ[Hb]	Change in [Hb]
ΔSO_2	Change in SO_2
T_SO_2_delay	Delay occurring in SO_2 recovery
T_SO_2_recovery	SO_2 recovery time
BHI	Breath-hold index
BHI_Δ[Hb]	BHI calculated using Δ[Hb]
BHI_ΔSO_2	BHI calculated using ΔSO_2
T_Bradycardia	Effective bradycardia duration

1. INTRODUCTION

Breath-hold diving (free diving or apnea diving) has been practiced for collecting sponges, pearls and seafood, for the recovery of sunken treasures or for military purposes all over the world for more than 2000 years. Although the usage of professional breath-hold divers has recently been replaced with SCUBA (Self Contained Underwater Breathing Apparatus) diving, it is still practiced as a competitive sports discipline and limits of human physiology are tested against greater depths.

Brain is one of the most sensitive organs to oxygen supply. Brain neither can shift to anaerobic metabolism like muscles when oxygen supply is not adequate nor its oxygen storing capacity is significant compared to its energy needs. Thus it requires a constant oxygen supply to maintain its functional and structural integrity. Hence specific mechanisms are necessary to guarantee the correct oxygenation levels.

It is known that diving birds or mammals achieve incredible dive depths and dive times without any loss of performance or without any structural damage. Some metabolic and physiologic adaptations along with special reflexes are developed for diving. While relatively shorter-duration divers such as Steller sea lions show signs of developed aerobic capacities similar to athletic terrestrial mammals of comparable size and have elevated concentrations of myoglobin in their skeletal muscle, long-duration divers like Weddell seals have a lipid based metabolism adapted for low levels of oxygen [1]. Also, recent comparison between mammalian species considered terrestrial and marine showed significant differences for brain globins (neuroglobin and cytoglobin) concentrations. These globin groups facilitates the oxygen transfer into brain tissues and providing protection against reactive oxygen and nitrogen groups [2]. Besides these adaptations, marine birds and mammals exhibits strong protective mechanisms known as diving response (or diving reflex) enabling them to endure conditions that can not be tolerated by terrestrial animals. Diving response mechanisms are aimed to reduce the oxygen uptake and conserve oxygen stores for vital organs during the dive.

2. THEORY

2.1 Diving Response

The aim of the diving response is to protect vital organs from harm, in other words to stay unharmed or even alive in extreme hypoxic and hypercapnic conditions. Similar mechanisms, also present in humans [3]. The human diving response consists of mainly bradycardia, decreased cardiac output due to reductions in heart rate and stroke volume, peripheral vasoconstriction, spleen contraction and increase in arterial blood pressure [3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. Previous studies showed that diving response has an oxygen conserving effect both at rest and during exercise in supplying the heart and brain with oxygen, while the peripheral oxygen stores were depleted. Smaller oxygen desaturation observed in subjects with more pronounced diving responses. Consequently a better diving response means better oxygen conservation and thus will enable longer apnea durations [6, 7, 8]. It has been observed that subjects with most significant cardiovascular responses have the slowest decline in arterial O_2 saturation [13]. Presence of an adaptive metabolic response is supported with the findings of a recent study. Increased tolerance to hypoxia and hypercapnia, a reduced level of blood acidosis and less production of oxygen free radicals in breath-hold divers during dynamic apnea clearly presents an adaptation as a consequence of repeated periods of apnea [13]. The presence of arrhythmias and large increases in blood pressure shows that the degree of adaptation in humans is inferior to diving animals [14]. A study on standard neuropsychological performance showed that breath-hold divers with up to 20 years of diving experience including some adverse neurological events, performed within the average range compared to norms [15].

2.1.1 Bradycardia

A smoothly developing bradycardia and continuously decreasing heart rate is described in diving animals. Starting with an initial tachycardia, diving bradycardia trend from human subjects are observed to be different than in animals [14]. While performing exercise during apnea, the diving response is powerful enough to suppress the heart rate increase demand due to exercise [6].

2.1.2 Decreased Cardiac Output

Circulatory adjustments like decreasing cardiac output and peripheral blood flow reduces O_2 uptake from lung to blood and preserves lung oxygen stores during apnea [7].

2.1.3 Peripheral Vasoconstriction

The changes in tissue perfusion parameters can not be explained solely by cardiac index changes [16]. Decreases in peripheral blood flow and increase in carotid artery blood flow during apnea suggests redistribution of the blood flow and supports the idea of peripheral vasoconstriction [6, 7]. A shift from aerobic to anaerobic metabolism has been demonstrated during prolonged dives in diving mammals [6]. In humans increase in blood lactate level probably due to a decrease in blood flow and oxygen delivery to muscles after apnea are reported [7, 14]. Lactate level is a sign of a shift from aerobic to anaerobic metabolism. Occurrence of anaerobic metabolism without an elevated metabolism can be counted as another proof of peripheral vasoconstriction.

2.1.4   Spleen Contraction

During periods of inactivity spleen contains 200 to 250 ml. of condensed red blood cells in an average human, which approximately correspond to 8% of total body red blood cells. This ratio is rather weak compared to horse or seal splenic ratio of 50%. Recent studies on spleen volume with a series of successive apneas showed that the splenic contraction starts with the first apnea and remains constricted for the rest of the   test and even for 1 hour after the apnea series. This suggests that splenic contraction in human occurs as a part of the diving response. When normal O_2 or CO_2 transport capacity is no longer adequate, addition of splenic red blood cells into circulation increases O_2 or CO_2 transport capacity of the blood and oxygen stores in the body and contributes to prolongation of successive apnea attempts. Contraction of spleen volume was greater in breath-hold divers than in non-divers [8, 17]. A comparison between breath-hold divers, intact non-divers and splenectomized persons showed that breath-hold divers performed longer apneas than intact non-divers, who in turn performed longer apneas than splenectomized persons, showing the contribution of spleen contraction to apnea performances [17, 18]. Higher Hb increase after apneas in breath-hold divers suggest a specific training effect due to regular apnea practice. Level of hypoxia, which can not be achieved during sub-maximal or even maximal exercise, may be required to evoke a greater splenic contraction [19].

2.1.5   Increased Arterial Blood Pressure

In normal conditions, at rest cerebral autoregulation is able to maintain cerebral blood flow over a wide range of cerebral perfusion pressures [20]. On the other hand during apnea, blood pressure increases as the systemic resistance increases [8]. This is considered as a defect in the human diving response since diving animals are able to maintain a steady blood pressure during dives [14]. Peripheral vasoconstriction is not matched by a vasoconstriction in carotid artery and in brain vessels, thus progressive increase in blood pressure ensures adequate oxygen delivery to most sensitive organs, the heart and the brain [21].

2.2   Breath-Hold Dive

We can differentiate three environmental phases in a breath-hold dive. These phases can be named as descent phase, underwater phase and ascent phase. Divers experience hyperoxic hypercapnia during the descent phase and during the time spent underwater of a breath hold dive and hypoxic hypercapnia during the ascent phase [22]. On the other hand, if we take another point of view, we can separate a breath-hold dive in to two physiological phases separated by a physiological break point; a relatively comfortable phase where the subject is at ease and a "struggle phase" where the subject experiences an urge to breath due to elevated arterial CO_2 tension, lowered O_2 tension and excited respiratory muscles [21].

2.2.1   Descent

For the descent phase the ambient pressure increases as the depth increases. As a result of this pressure increase partial pressure of the gases within the lungs and blood increases thus hyperoxic and hypercapnic conditions occur.

2.2.2   Underwater

With the time spent underwater amount of O_2 decreases but due to large ambient pressure, partial pressure of O_2 perceived by baroreceptors remains higher and hypercapnia increases further as CO_2 accumulates if the physiological breakpoint is not achieved. When the physiological breakpoint achieved at depth it signals trouble for the diver for reasons which will be explained in the ascent phase.

2.2.3   Ascent

At the end of the dive as the diver ascends towards the surface the ambient pressure decrease down to the surface pressure. For a subjectively short dive depending on the condition of the diver, even with the decrease in pressure, O_2 tension remains normoxic; does not reach hypoxic conditions.

However if the dive was long enough, in other words physiological breakpoint is reached at the bottom or during the ascent, due to depletion of O_2 stores and decrease in pressure, condition changes from hyperoxia to hypoxia or even anoxia which may lead to unconsciousness and sometimes death due to drowning. The phenomenon is called "shallow water blackout". On the other hand hypercapnic condition remains due to accumulation of CO_2 even though the partial pressure decreases [15].

2.3   Apnea

The human diving response can be triggered to some extend by apnea [6, 7]. Dry breath holds can create a similar environment to breath-hold dives except the effect of immersion into water. After a maximal dry breath hold, partial pressure of alveolar oxygen may become lower than 30 mmHg and partial pressure of alveolar carbon dioxide can go up greater than 50 mmHg [5]. Hypoxemia and hypercapnia are both potent cerebral vasodilators. In addition, systemic blood pressure increases caused by the apnea can result in passive increases in cerebral blood flow. This increase is not counteracted by cerebral autoregulation because they are instantaneous or just because autoregulatory vasoconstriction is suppressed by vasodilatory effects of hypercapnia and hypoxemia [23].

2.3.1 Oxygen

Hypoxia, hyperoxia, normoxia and anoxia describes the oxygenation condition of the body where hypoxia means smaller concentration of O_2 and hyperoxia means larger concentration of O_2 with respect to normoxia which is the normal condition. Anoxia refers to a low level of O_2 tension where unconsciousness occurs. Cerebral blood flow mostly depends on O_2 and CO_2 levels. Hypoxia has been shown to increase cerebral blood flow while hyperoxia reduces cerebral blood flow [24]. Studies on exposure hypoxia indicate that increases in cerebral blood flow and cerebral blood flow velocity are aiming to maintain O_2 delivery to brain. Increase in cerebral blood flow depends on the severity of hypoxia and relation found to be exponential [25]. Thus brain O_2 supply is protected even with severe degrees of hypoxia [26]. Even though cerebral blood flow successfully protects the brain to some extend, it is reasonable that the O_2 saturation decreases with depletion of O_2 stores [27].

2.3.2 Carbon Dioxide

Hypocapnia, hypercapnia and normocapnia refers to the carbon dioxide tension of the body where normoxia is the normal level and hypocapnia and hypercapnia are lower and higher levels respectively. Hypercapnia has been found to induce cerebral vasodilation and thus increase cerebral blood flow and cerebral blood volume. Increases in cerebral blood flow and cerebral blood volume are not the same, cerebral blood flow has been found to increase more and it is caused mainly by an increase in vascular blood velocity [28, 29]. It is proven that the ventilatory response to hypercapnia is diminished in breath-hold divers [10, 21]. Cerebrovascular reactivity of breath-hold divers found to be unchanged, and normal cerebrovascular response to hypercapnia provides maintained blood flow in the presence of adequate vasodilators [10].

2.3.3 Hypercapnia vs. Hyperoxia

It has been reported that hypercapnia causes an increase in cerebral blood flow even under hyperoxic conditions, thus it can be concluded that the dominant chemical stimulus controlling cerebral blood flow is hypercapnia [23, 28]. It has been found that the cerebral blood flow is increased as early as 20 seconds after the onset of the apnea, being to early for hypoxia to occur and/or trigger any regulatory mechanisms, thus, partial pressure of CO_2 is defined to be the primary regulator for the increase in cerebral blood flow during apnea [23].

2.3.4 Easy going Phase

In this phase, there is little discomfort, O_2 stores are not yet depleted and CO_2 tension is not elevated high enough to cause trouble. Bradycardia and peripheral vaso-constriction is effective in this phase. The effect of involuntary respiratory movements on the intrathoracic pressure is too insignificant to have an effect on cardiovascular system [21].

2.3.5 Urge to Breath

Breath-hold is a voluntary act but breakdown of the act seems to occur involuntarily since normal subjects cannot hold breath to unconsciousness. Normally a strong mechanism overrides voluntary breath-holding.

Studies on breath-hold divers suggest a blunted ventilatory response against hypoxia despite significant peripheral and central stimuli. It is clear that the absence of urge to breathe is good for the comfort of the breath-hold diver but it is argued whether it is also advantageous from a safety point of view since it is known that elite breath-hold divers occasionally forget to breathe after long dives or apneas during practice or record attempts [9]. Independent from the O_2 stores in the body or the

oxygen consumption, breaking point occurs at a point approximate to a given alveolar gas composition depending on the subject [5]. Thus the breath hold time is related to the O_2 stores in the body and inversely related to O_2 consumption rate.

Breakdown of breath-hold cannot be explained by single variable hypotheses based on arterial pressures of blood gases or lung volume or carotid arterial chemoreceptors. Hypotheses based on combined gas pressures and lung volume has to be considered but multivariable hypotheses are too difficult to test scientifically due to complexity [30].

2.3.6 Hyper Ventilation

Hyper ventilation causes a small amount of increase in body O_2 stores but mainly it results in reduction of CO_2 in the circulation and accumulated in the body. By retarding the chemical stimulus triggered by accumulation of CO_2, hyper ventilation can extend the dive duration. But in cases where the diver does not know or tries to go beyond his or her own limits this may become the last dive for reasons explained earlier in the ascent of a breath-hold dive.

2.4 Breath-Hold Divers vs. Non-Divers

A recent study showed that skeletal muscle oxygen desaturation occurs earlier than the arterial blood in both breath-hold divers and non-divers, providing further support that the oxygen stores are preserved for vital organs with the diving response. It has been also found smaller muscle O_2 desaturation in breath-hold divers than non-divers for apnea durations maximal for non-divers while arterial saturation did not changed, which suggest muscular adaptations to hypoxia in breath-hold divers [8]. Comparative studies showed that diving response mechanisms are accentuated in breath-hold divers [5, 14, 31].

Findings for intact cognitive functioning in breath-hold divers support the possibility of an adaptive response to apneic conditions [15]. Since elite breath-hold divers are able to sustain breath-holds over 8 min. and endure dives to 200 meter it is important to investigate adaptive mechanisms. Thus the aim of this study was to investigate the effects of a maximal static breath-holding on systemic oxygen saturation, heart rate variability and brain perfusion in breath-hold divers. We hypothesized that cerebral blood flow during maximal breath-holds is increased more in elite divers versus non-divers in order to enhance brain protection and that peripheral vasoconstriction is enhanced in these subjects.

3. METHODS

3.1 Subjects

Three groups of subjects participated in the study. Group1 consists of 6 experienced free divers (1 female, 6 male), Group2 consists of 16 beginner free divers (5 female, 11 male) and Group3 is the control group of 9 non-divers (5 female, 4 male). All subjects were university students with 23.9 ± 2.77 age and they were healthy at the time of the experiment. Experienced free divers and beginner free divers were members of BÜSAS (Boğaziçi Üniversitesi Sualtı Sporları Kulübü) and they followed a 3 months training program before the tests.

3.2 Experimental Setup

Brain oxygenation, body oxygenation and heart rate data are recorded for each subject. Cerebral hemodynamic data are recorded using NIROXCOPE with two linearly arranged probes from the forehead of the subjects as shown in **Figure 3.1**. Breath hold task is supposed to cause a systematic perturbation in cerebral cortex. This perturbation is not regional or localized; therefore the forehead was a suitable position for the probe. Two probes are placed about 2 cm beside the middle line and 2 cm above the eyebrows. Location of the probes is chosen in order to avoid the sinuses and hairs. Also the skin on the selected area is very thin and poorly perfused. To prevent external light from interfering with the measurements back of the probe is covered with a black plastic band as shown in **Figure 3.2**. Heart rate and body oxygenation data are recorded with Masimo Rad-9$^{\text{TM}}$, Signal Extraction Pulse Oximeter$^{\text{TM}}$ from the middle finger of the non dominant hand of the subjects. The finger bearing the Pulse Oximeter was positioned at the heart level and kept at the same level for the duration of the study.
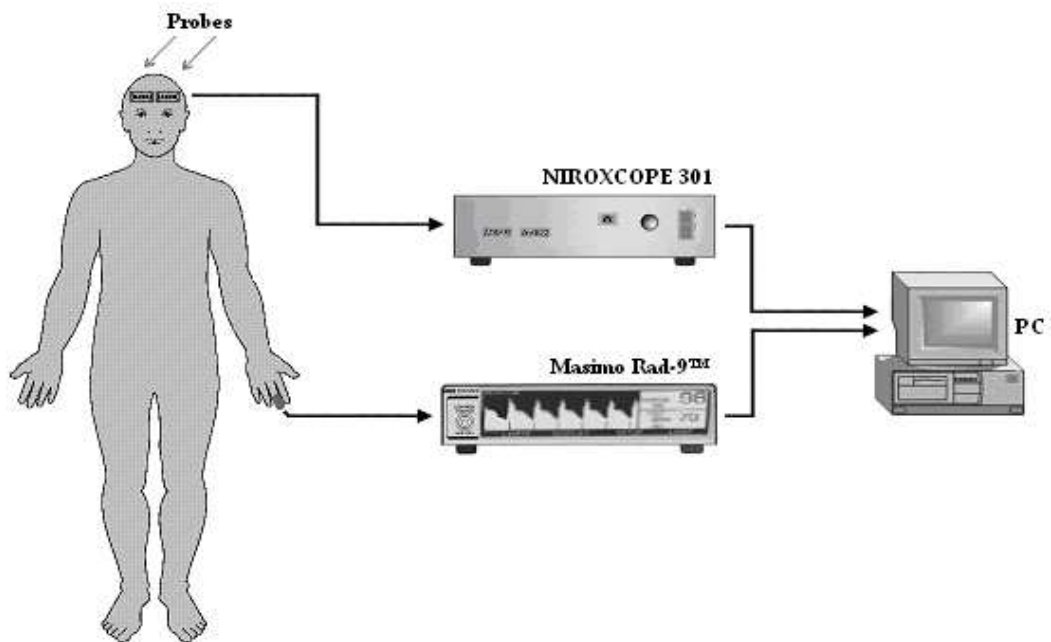
Figure 3.1 Experimental setup.
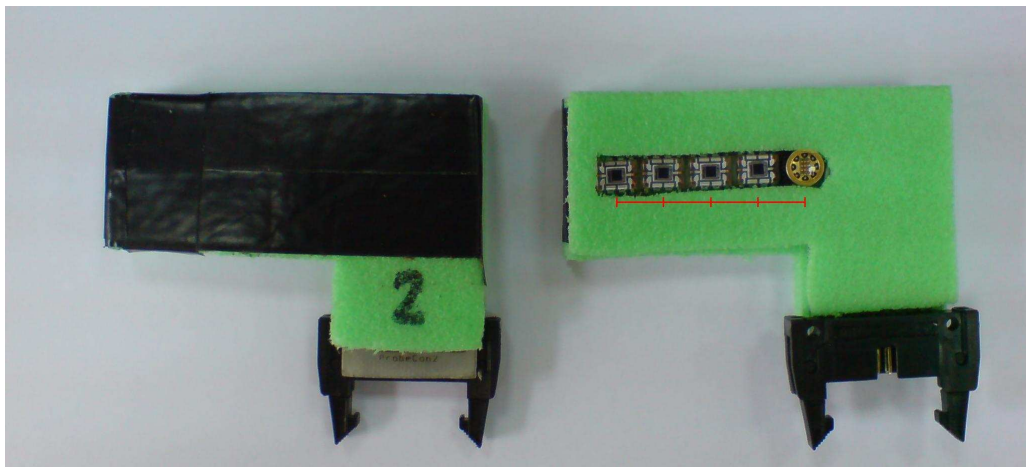
Figure 3.2 Probes. Front view (right), detectors with 1 cm. incremental separation for each partition of the scale. Back view (left), black rubber band covering the back of probes.

3.3 Experimental Protocol

All experiments were performed in the laboratory where the temperature was controlled by air conditioning. Subjects relaxed in supine position, taking steady breaths until their heart rates stabilized and ensure blood mixing as stated in literature [19]. The task was explained carefully to the subjects before the beginning of the experiment.

Task consists of four maximum breath-holds and two minutes rest periods before and after each breath hold. Task begins with an initial rest period and ends with a final rest period as shown in **Figure 3.3**. Ten to Fifteen seconds before the end of the rest period subjects took two slow and deep breaths and started the breath-hold period. The total task takes up to twenty five minutes depending on the subject's breath-hold durations.
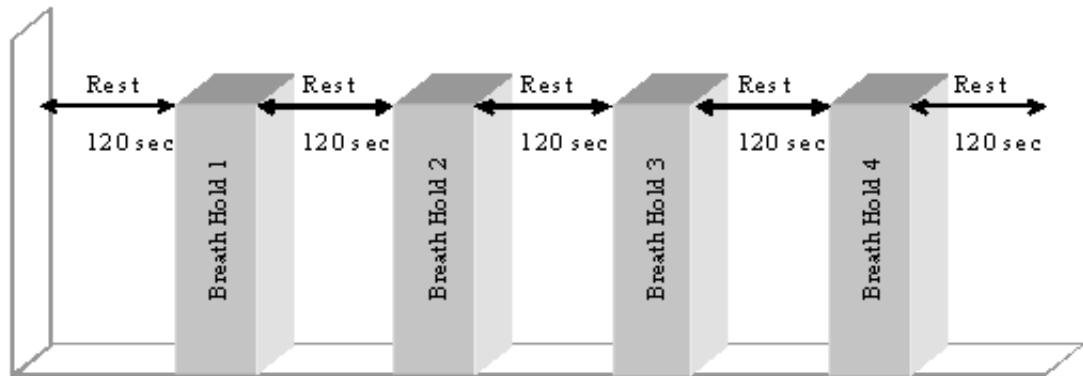
Figure 3.3 Breath-hold task.

3.4 Analysis

The data from NIROXCOPE is used to calculate the relative changes in hemoglobin concentration ([Hb]) signals according to the Beer Lambert Law with an average sampling rate of 1.6 Hz.

Data from Masimo Rad-9$^{\text{TM}}$ are used to plot Heart Rate signals and Systemic Oxygen Saturation (SO$_2$) Signal. The results were automatically calculated and recorded by the device with 2 seconds resolution.

All data, measured and calculated were analyzed for statistical significance in the MATLAB® environment. 3 kinds of test performed for each parameter. A one way ANOVA for comparing consecutive breath-holds within groups, another one way ANOVA for difference between groups for breath hold tasks and finally a two way ANOVA applied on whole set for a general view.
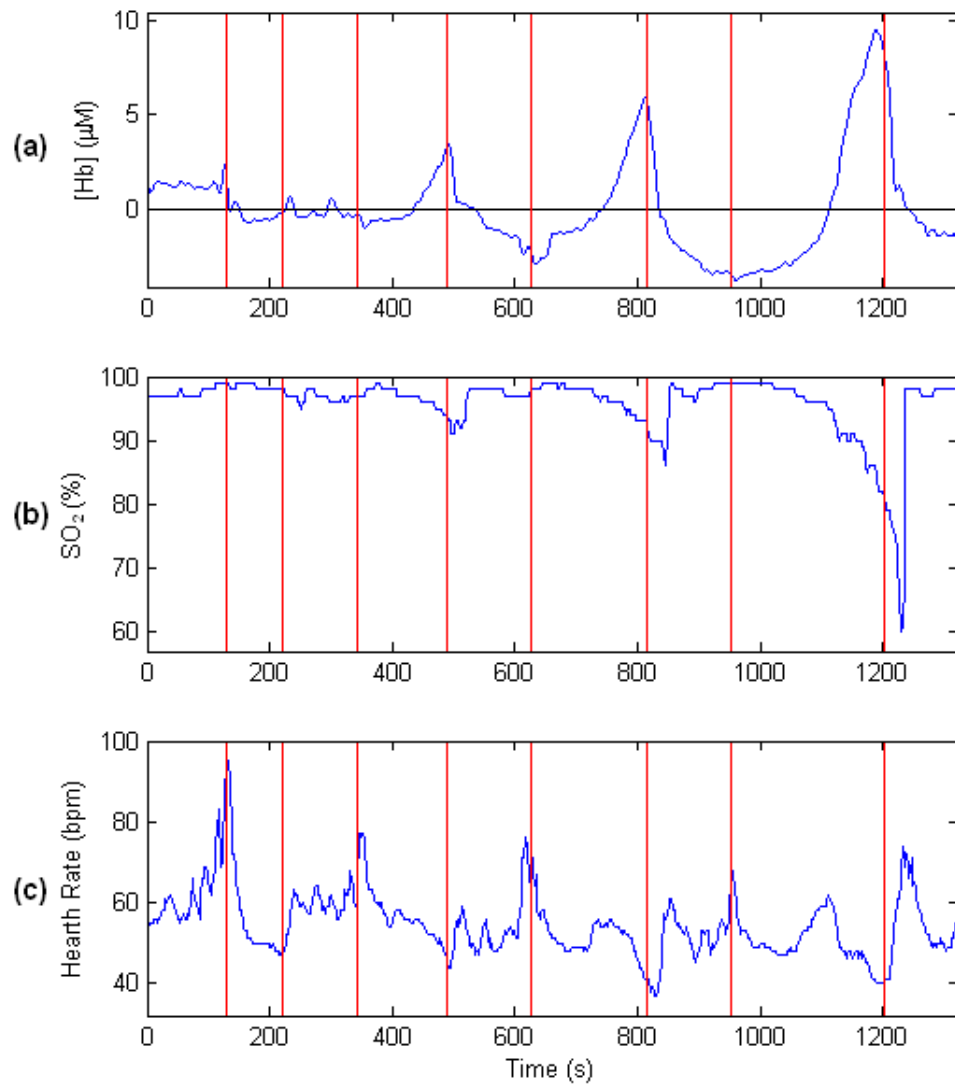
Figure 3.4 A sample data during the task from an experienced diver. **(a)** [Hb] signal, **(b)** SO_2 signal and **(c)** Heart Rate signal. Vertical lines mark the beginning and ending times of breath-holds.

3.5 Data Processing and Curve Fit

Data from the subjects contained noise and some artifacts due to motion, breathing and heart rate. A program developed in the MATLAB® environment is used to eliminate outliers from the data. Furthermore the data are filtered with a fourth degree Butterworth low pass filter having 0.08 Hz as cut off frequency, again in the MATLAB® environment. Finally in order to eliminate the baseline drifts, the linear shift is removed from the data via "detrend" function of the MATLAB® environment.

The [Hb] data is separated into nine periods, five rests including the initial rest and four breath holds and periods are tagged by markers. Breath-hold times are calculated by the difference between markers signaling the beginning and end of breath-hold periods.

The characteristic of the [Hb] signal observed to be in the form of an exponential increase during breath-holds and an exponential decrease during rest periods. Curves in the form $Ae^{Bx} + C$ and $Ae^{-Bx} + C$ are fitted to breath-hold and rest data accordingly using Curve Fitting Toolbox in the MATLAB® environment where A is the amplitude parameter, B is the time parameter and C is the offset parameter. Some sample data for breath-hold period and rest period and related fits can be seen in **Figure 3.5**.

Change in hemoglobin concentration (Δ[Hb]) is calculated using the fits, this is the difference between the baseline value at the beginning of the breath-hold and the maximum value reached at the end of breath-hold.

Three recovery times of [Hb] signal are also calculated using fits. Recovery to 10%, 3% and 1% of the difference are calculated in order to investigate the recovery period.

Systemic oxygen saturation keeps decreasing after the end of breath-hold period representing a delay in recovery, thus the change in SO_2 (ΔSO_2) is calculated as the average SO_2 during rest minus the minimum value reached after the breath-hold. Delay time in SO_2 (T_SO_2_delay) is the time difference between the marker at the end of breath-hold and inflection point of signal where increase starts. Time of SO_2 recovery (T_SO_2_recovery) is calculated as the time difference between the start of the SO_2 increase and the point where SO_2 reaches its maximum.

Two breath-hold indices calculated with our data according to an example in literature [33].

First breath-hold index BHI_Δ[Hb] for each breath-hold is calculated using the
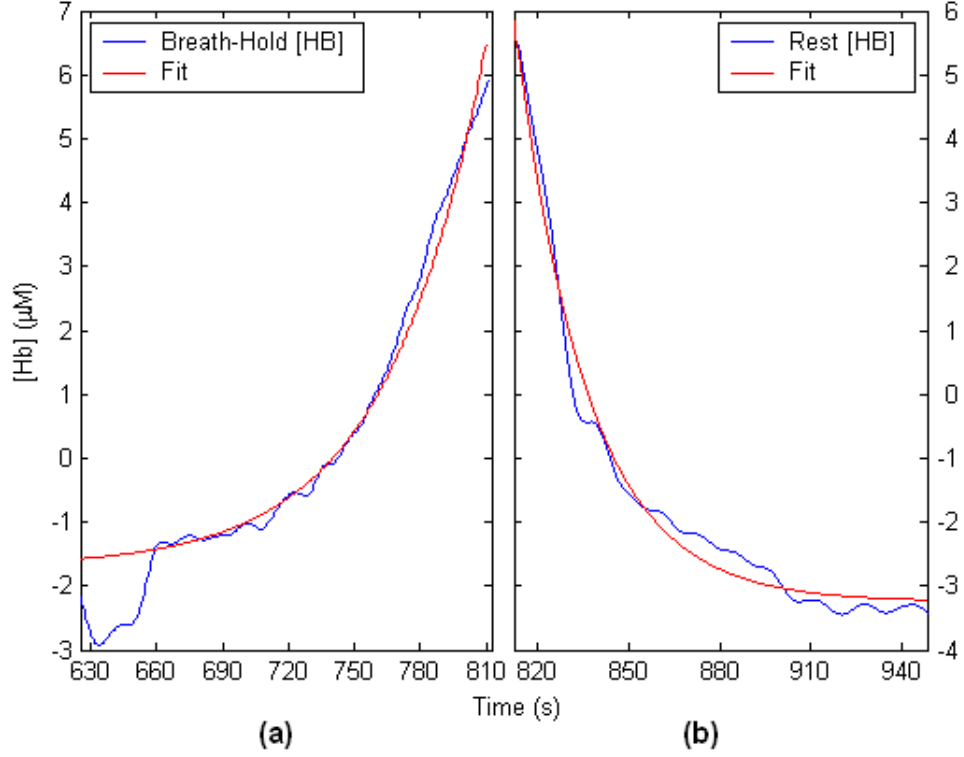
Figure 3.5 A sample of [Hb] data during breath-hold **(a)** and during rest **(b)** and their related fits where A=1.05, B=1.225, C=-1.7 and A=0.52, B=-1.706 and C=-3.244 respectively.

increase in [Hb] and dividing by the duration of the breath-hold as shown in **Equation 3.1**.

$$BHI\_\Delta[Hb] = \frac{\Delta[Hb]}{\Delta T} = \frac{[Hb]_{final} - [Hb]_{initial}}{T_{Breath-hold}} \tag{3.1}$$

Second breath-hold index BHI_ΔSO_2 for each breath hold is calculated by dividing the decrease in Systemic oxygen saturation by the duration of the breath-hold as shown in **Equation 3.2**.

$$BHI\_\Delta SO_2 = \frac{\Delta SO_2}{\Delta T} = \frac{SO_{2\ final} - SO_{2\ initial}}{T_{Breath-hold}} \tag{3.2}$$

Expectance for bradycardia reflects a continuous decrease in heart rate, but studies in literature are generally performed with sub-maximal breath-holds. In our study we did not observe bradycardia for the duration of the breath hold except for a

few very experienced subjects. In a study, experimenter was able to abolish the brady-cardia by disturbing the subject and concluded that stress may overcome responses to breath-hold [33]. Thus we assume that bradycardia ceases due to loss of concentration, increase in subjective discomfort since discomfort cause observable stress in the subject. Thus we calculated the bradycardia time (T_Bradycardia) as the time difference between the onset of the apnea and the time where the subject's heart rate reached it minimal value for each breath-hold.

4. RESULTS

As a primary concern we investigated whether there is a significant difference in the breath-hold performances between male and female subjects within groups. 1 way ANOVA was performed on breath-hold times, Change in [Hb] during breath-holds and breath-hold indices within groups revealed that there is no significant difference between male and female subjects.

In Figures and Tables, Group1 is the most experienced group, Group2 is the group of beginner free divers and Group3 is the control group. As for the statistical analysis, [1] means statistically different than Group1, [2] means statistically different than Group2 and [3] means statistically different than Group3 for the p values given by one way ANOVA performed within groups on each breath-hold pairs. Vertical and horizontal p values are results of two way ANOVA using ANOVAN function of the MATLAB® environment, while vertical p value shows the significance between groups and horizontal p value shows the significance of consecutive breath-hold tasks.

The aim of performing four consecutive breath holds was to see the short term adaptation and we expected to see an increase in breath hold durations as the subjects attempt second, third and fourth breath holds. **Table 4.1** shows the mean breath-hold durations of each group, standard deviations and statistical significances. Vertical p value ($<5 \times 10^{-15}$) and horizontal p value ($=2.3 \times 10^{-11}$) shows that there are strong significant differences between groups and consecutive breath-holds. Increase in breath-hold durations and also the increase rates can be seen in **Figure 4.1**. We can see that subjects within each group adapts to the consecutive breath-holding by an increase in breath-hold duration. Group1 and Group2 show an increase with each breath-hold while Group3 subjects seem to reach a plateau after 2nd hold. Adaptation to consecutive breath-holding can be visualized by using slopes. The higher the slope the better the adaptation of the group, hence Group1 outperforms the other groups as expected.

Table 4.1
Breath-hold times in form of averages $\pm$ standard deviations in seconds.

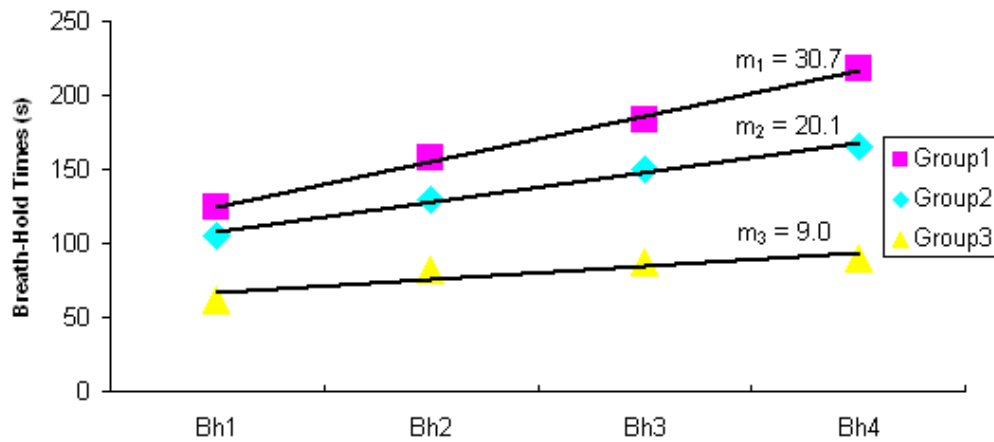| Bh Times	Bh1	Bh2	Bh3	Bh4	
Group1	124.17 $\pm$ 20.30 [3]	157.75 $\pm$ 14.53 [2,3]	183.86 $\pm$ 20.76 [2,3]	217.89 $\pm$ 49.07 [2,3]	$p<5\times10^{-15}$
Group2	105.5 $\pm$ 30.52 [3]	128.85 $\pm$ 28.32 [1,3]	149.62 $\pm$ 34.07 [1,3]	165.68 $\pm$ 42.05 [1,3]	
Group3	61.21 $\pm$ 14.91 [1,2]	81.89 $\pm$ 10.82 [1,2]	86.8 $\pm$ 20.11 [1,2]	89.66 $\pm$ 28.01 [1,2]	
	$p=2.3\times10^{-11}$				

Figure 4.1 Mean breath-hold times of groups for four consecutive breath-holds.

Increase in [Hb] is the result of breath-hold. As the body consumes oxygen the percentage of HbO_2 decreases and there is more Hb. On the other hand as the CO_2 level increases, Hb's attach to CO_2's in order to adjust the free CO_2 concentration in the blood thus adjusting the pH level of blood. In **Table 4.2** we have mean values clearly showing the difference in changes in [Hb] between groups for consecutive breath holds. There is a stronger statistical difference between groups than the the statistical difference between consecutive breath-holds which is also significant. Strength of significance can be seen in vertical p value ($=1.2\times10^{-14}$) and horizontal p value ($=2.8\times10^{-7}$). In **Figure 4.2** we see the trend of change in [Hb] along with average increase values for each breath-hold. Increasing values of [Hb] agree with literature [33]. We can observe the increase in performance for Group1 and Group2 while Group3 have almost a horizontal trend where a greater slope means a better performance increase during consecutive breath-holds.

Table 4.2
Changes in [Hb] in form of averages $\pm$ standard deviations in μM.

Δ[Hb]	Bh1	Bh2	Bh3	Bh4	
Group1	3.05 ± 2.30 [3]	4.68 ± 3.12 [3]	6.91 ± 3.95 [2,3]	9.96 ± 4.10 [2,3]	p<1.2x10⁻¹⁴
Group2	1.91 ± 1.23 [3]	2.97 ± 1.57 [3]	3.49 ± 1.22 [1,3]	4.86 ± 2.28 [1,3]	
Group3	0.99 ± 0.48 [1,2]	1.18 ± 0.58 [1,2]	1.25 ± 0.58 [1,2]	1.58 ± 0.70 [1,2]	
	p=2.8x10⁻⁷				
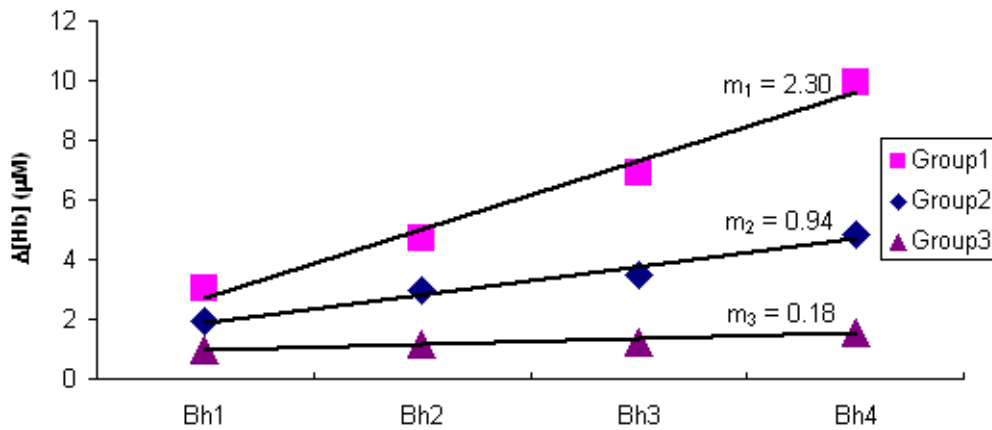
Figure 4.2 Average change in [Hb] of groups for four consecutive breath-holds.

Breath hold index for hemoglobin concentration change (BHI_Δ[Hb]) calculated from **Equation 3.1**, was calculated as the ratio of total change in [Hb] to breath-hold duration. In **Figure 4.3** we can see a comparison of BHI_Δ[Hb] of groups. Once again the greater performance increase can be seen in Group1. Group3 shows no sign of increase with a horizontal trend. Group2 stands between the two groups as expected. Numerical and statistical results along with standard deviations can be seen in **Table 4.3**. Vertical p value (<5x10^{-5}) being smaller than the horizontal p value ($=2.6$x10^{-3}) shows that the statistical difference between groups is greater than the statistical difference between consecutive breath-holds.

Systemic Oxygen Saturations of subjects should always be lower than the Oxygen supply to the brain since the body transfer the blood to the brain in order to

Table 4.3
Breath-hold index in form of averages $\pm$ standard deviations in $\mu M/s$.

BHI_Δ[Hb]	Bh1	Bh2	Bh3	Bh4	
Group1	0.023 ± 0.015	0.029 ± 0.016 [3]	0.037 ± 0.018 [2,3]	0.045 ± 0.013 [2,3]	p<5x10⁻³
Group2	0.019 ± 0.012	0.023 ± 0.010 [3]	0.024 ± 0.009 [1,3]	0.029 ± 0.012 [1,3]	
Group3	0.016 ± 0.007	0.015 ± 0.007 [1,2]	0.014 ± 0.005 [1,2]	0.018 ± 0.005 [1,2]	
	p=2.6x10⁻³				
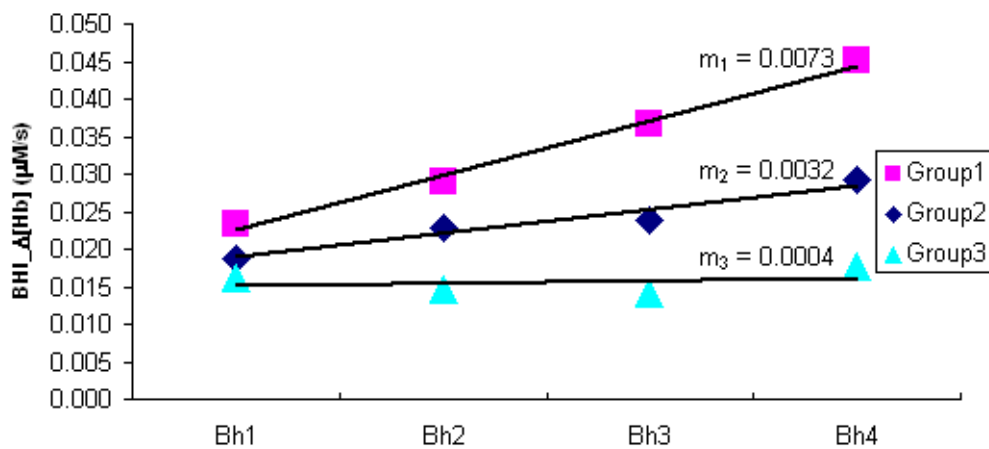
Figure 4.3 Mean BHI_Δ[Hb] of groups for four consecutive breath-holds.

protect it. In **Table 4.4** we can see the change in Systemic Oxygen Saturation for consecutive breath-holds. Vertical p value ($<5\text{x}10^{-5}$) and horizontal p value ($=1.2\text{x}10^{-4}$) shows that there is significant difference between groups and consecutive breath-holds. A graphic representation of average Systemic Oxygen Saturation decrease values can be seen in **Figure 4.4**. We observe a small decrease for Group3, a greater decrease for Group2 and an even greater decrease in Group1. While trend for Group3 and Group2 are linear, the trend for Group1 is parabolic meaning a greater decrease and a better adaptation to hypoxia.

Breath hold index for systemic oxygen saturation change (BHI_ΔSO_2) calculated from **Equation 3.2**, was calculated as the ratio of total change in SO_2 to breath-hold duration. In **Figure 4.5** we can see a comparison of BHI_ΔSO_2 of groups. We

Table 4.4
Change in SO_2 in form of averages $\pm$ standard deviations as % change.

ΔSO_2	Bh1	Bh2	Bh3	Bh4	
Group1	-8.46 ± 4.44 [3]	-11.18 ± 5.79 [3]	-16.89 ± 7.96 [3]	-34.89 ± 22.69 [2,3]	$p<5\text{x}10^{-5}$
Group2	-6.66 ± 6.32 [3]	-9.05 ± 8.90	-11.26 ± 9.05 [3]	-14.93 ± 10.79 [1,3]	
Group3	-1.93 ± 1.29 [1,2]	-2.82 ± 1.36 [1]	-2.71 ± 1.17 [1,2]	-3.27 ± 1.61 [1,2]	
	$p=1.2\text{x}10^{-4}$				
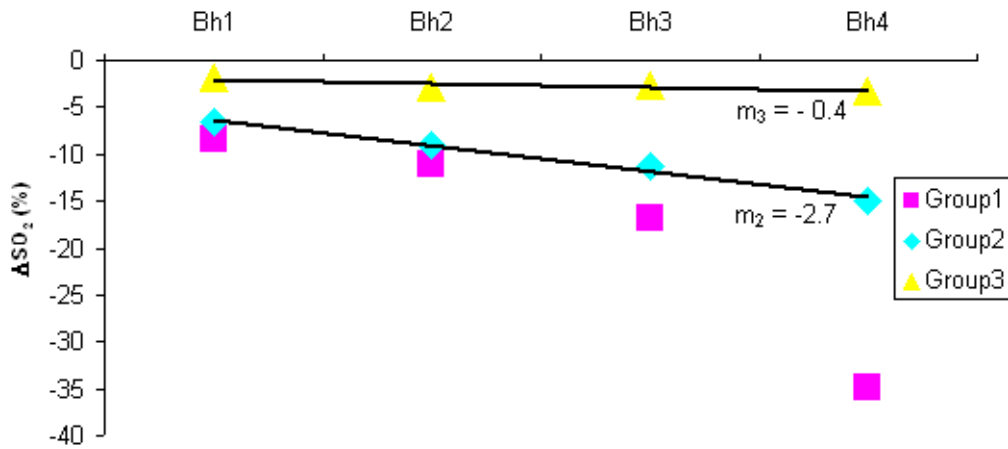
Figure 4.4 Average SO_2 decrease of groups for consecutive breath-holds.

can clearly see the increase of performance with consecutive breath-holds in Group1 and Group2 while the change is greater in Group1. Group3 shows no sign of performance increase as indicated by a horizontal trend. Numerical and statistical results with standard deviations can be seen in **Table 4.5**. Vertical p value ($<5\text{x}10^{-5}$) shows that there is significant difference between groups while horizontal p value ($=0.0128$) signals a weaker statistical difference between consecutive breath-holds.

As explained in the methods section, we did not observe bradycardia in line with breath-hold times. Probably associated with the initiation struggle phase, subject's heart rate increased. We calculated time to reach minimum heart rate as achieved bradycardia duration. In **Table 4.6** mean values for bradycardia durations and standard deviations are given along with statistical significances. Vertical ($=5.6\text{x}10^{-7}$) and

Table 4.5
Mean BHI_ΔSO_2 in form of averages $\pm$ standard deviations as % change/s.

BHI_ΔSO_2	Bh1	Bh2	Bh3	Bh4	
Group1	-0.07 ± 0.03 [3]	-0.07 ± 0.03 [3]	-0.09 ± 0.04 [3]	-0.15 ± 0.08 [2,3]	p<5x10⁻⁵
Group2	-0.06 ± 0.04	-0.07 ± 0.05	-0.07 ± 0.05 [3]	-0.09 ± 0.05 [1,3]	
Group3	-0.03 ± 0.02 [1]	-0.04 ± 0.02 [1]	-0.03 ± 0.02 [1,2]	-0.04 ± 0.02 [1,2]	
	p=0.0128				
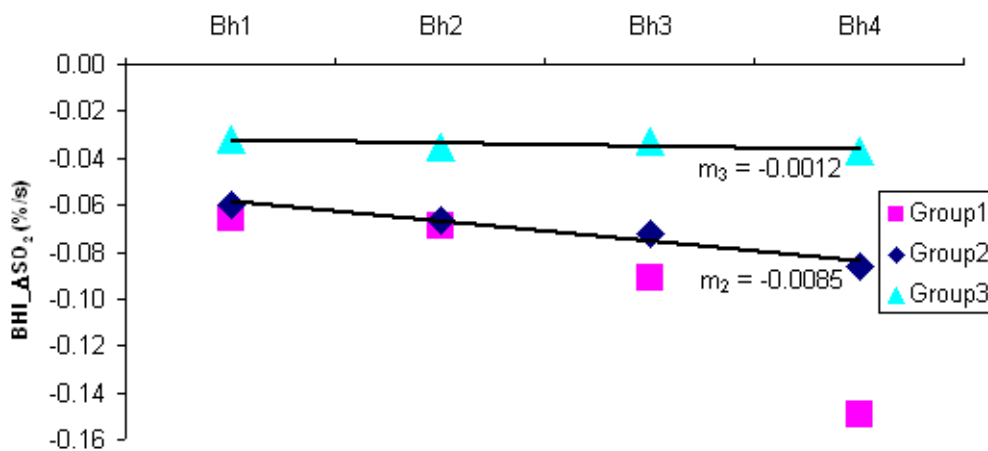
Figure 4.5 Mean BHI_ΔSO_2 of groups for consecutive breath-holds.

horizontal ($<5x10^{-5}$) p values shows that both factors, groups and consecutive breath-holds are statistically significant but the groups have stronger significance. The trend over consecutive breath-holds is shown in graphics in **Figure 4.6**. For the last and longer breath-hold average apnea time calculated to be 50%, 61% and 80% of breath hold duration respectively in Group1, Group2 and Group3. Longer bradycardia is a sign of better diving response. Bradycardia times do not change much despite little increase in breath-hold times in Group3.

Calculated mean recovery times of [Hb] signal given in **Table 4.7**. Recovery to 10%, 3% and 1% are calculated in order to obtain a trend of recovery instead of one definite recovery point. Values are given as averages and standard deviations. Recoveries seem to follow a decreasing trend but test results display no significant

Table 4.6
Mean bradycardia durations in form of averages ± standard deviations in seconds.

T_Bradycardia	Bh1	Bh2	Bh3	Bh4	
Group1	62 ± 31	102 ± 58 [3]	117 ± 56 [3]	174 ± 85 [2,3]	p=0
Group2	62 ± 34	82 ± 45 [3]	90 ± 53 [3]	101 ± 67 [1,3]	
Group3	43 ± 22	42 ± 25 [1,2]	47 ± 26 [1,2]	45 ± 40 [1,2]	
	$p < 5 \times 10^{-3}$				
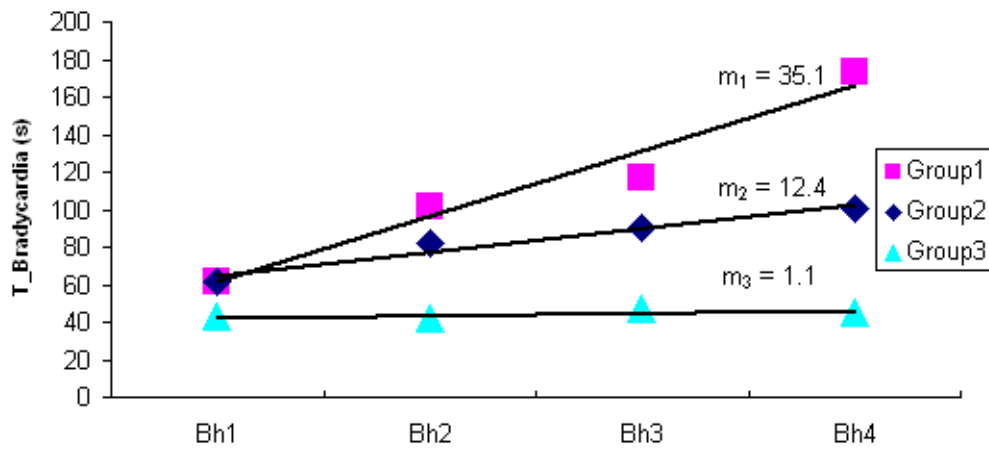
Figure 4.6 Average bradycardia times of groups for consecutive breath-holds.

difference between groups and breath-holds for all three recoveries. Lack of significant difference signals similar perfusion properties for all three groups.

Delay occurring in SO_2 recovery and SO_2 recovery times are given in **Table 4.8**. Statistical test resulted in no significant difference between groups or breath-holds.

4.1 Curve Fitting (Multiple Regression Analysis)

Fits were evaluated for [Hb] signal to obtain the A, B and C values for each breath hold according to **Equation 3.1** and **Equation 3.2**. Parameter A being related with amplitude is not accounted for since amplitude was already considered as d[Hb]

Table 4.7
Average recovery times to 10% **(A)**, 3% **(B)** and 1% **(C)** for [Hb] signal of fits for rest periods in form of averages ± standard deviations in seconds.

T_Rec 10%	Bh1	Bh2	Bh3	Bh4
Group1	47.2 ± 10.8	53.9 ± 8.7	48.4 ± 16.4	34.5 ± 9.0
Group2	46.6 ± 22.8	44.3 ± 25.4	40.6 ± 14.4	28.3 ± 7.7
Group3	54.9 ± 28.4	49.8 ± 23.0	43.8 ± 26.4	33.1 ± 19.7

(A)

T_Rec 3%	Bh1	Bh2	Bh3	Bh4
Group1	67.2 ± 16.3	78.0 ± 11.8	70.5 ± 22.5	51.3 ± 14.5
Group2	64.5 ± 27.8	61.2 ± 30.0	58.9 ± 18.6	41.7 ± 11.7
Group3	70.4 ± 29.6	69.1 ± 29.2	57.9 ± 32.2	45.7 ± 25.5

(B)

T_Rec 1%	Bh1	Bh2	Bh3	Bh4
Group1	83.5 ± 19.4	96.8 ± 12.5	88.2 ± 25.3	65.8 ± 17.9
Group2	77.4 ± 28.7	74.0 ± 30.9	74.3 ± 20.0	53.7 ± 15.3
Group3	81.3 ± 29.1	82.7 ± 31.2	68.0 ± 33.9	55.9 ± 28.1

(C)

Table 4.8
Average T_SO_2_delay times **(A)** and T_SO_2_recovery times **(B)** in form of averages ± standard deviations in 1/s units.

T_SO_2_delay	Bh1	Bh2	Bh3	Bh4
Group1	24.9 ± 6.6	21.3 ± 7.5	20.6 ± 7.0	21.6 ± 5.0
Group2	22.6 ± 10.1	23.1 ± 9.5	21.9 ± 12.8	23.0 ± 10.8
Group3	27.8 ± 10.7	26.9 ± 9.2	22.8 ± 5.8	20.0 ± 6.3

(A)

T_SO_2_recovery	Bh1	Bh2	Bh3	Bh4
Group1	7.9 ± 3.0	10.6 ± 6.2	9.1 ± 2.5	9.3 ± 3.3
Group2	10.3 ± 6.9	9.4 ± 4.8	9.8 ± 3.9	9.6 ± 4.8
Group3	9.3 ± 8.2	9.6 ± 5.6	12.7 ± 10.3	11.3 ± 5.7

(B)

calculations. Parameter C is ignored to since it gives only a baseline value which is already modified by detrending the data. On the other hand parameter B was investigated since it is related to recovery time thus can give an idea about perfusion properties. In **Table 4.9** parameter B values for breath-hold periods and rest periods

are given as averages and standard deviations. There is no significant difference between groups and breath-holds, supporting the idea of normal perfusion properties for all groups.

Table 4.9
Average B values of fits for breath-hold periods **(A)** and rest periods **(B)** in form of averages ± standard deviations in 1/s units.

B_Bh	Bh1	Bh2	Bh3	Bh4
Group1	1.16 ± 0.42	0.99 ± 0.11	1.07 ± 0.18	1.05 ± 0.11
Group2	1.22 ± 0.65	1.32 ± 0.54	1.00 ± 0.35	1.07 ± 0.31
Group3	1.19 ± 0.81	0.98 ± 0.52	0.97 ± 0.32	1.25 ± 0.69

(A)

B_Rec	Bh1	Bh2	Bh3	Bh4
Group1	-1.71 ± 0.4	-1.60 ± 0.33	-2.04 ± 0.74	-2.71 ± 0.76
Group2	-1.22 ± 0.65	-1.32 ± 0.54	-1.00 ± 0.35	-1.07 ± 0.31
Group3	-1.19 ± 0.81	-0.98 ± 0.52	-0.97 ± 0.32	-1.25 ± 0.69

(B)

5. DISCUSSION

In different studies subjects were asked to perform sub-maximal or maximal breath-holds depending on the aim of the study. We preferred to perform maximal dry breath-holds for our study. If we had chosen to perform sub-maximal breath-holds, determining the breath-hold duration would be one major problem since shorter breath-hold times would not be long enough to cause significant responses in experienced breath-hold divers or on the other end, longer breath-holds would be impossible to be performed by the control group. Hence we chose maximal breath holds to challenge each subject with his or her own limits.

A previous study found increasing breath-hold times with consecutive breath holds [19, 30]. Our findings are in accordance with this. We observed increase in breath-hold times in all the three groups. In addition we also found that the increase was greater in groups with longer dive experience. The increase was 46% for Group3, 57% for Group2 and even greater, 75% for Group1. Increase in breath-hold times and the greater increase in breath-hold divers suggest that apnea training has provided an adaptation to prevent brain damage for longer durations in hypoxic and hypercapnic conditions.

As for d[Hb] values for consecutive holds we expected an increase in all groups [34]. The change increased with increasing breath-hold duration and the relation we found was exponential. Similar [Hb] signal pattern was previously observed by other researchers [33]. The increase in [Hb] is likely to occur due to spleen contraction [19] and cerebral blood flow increase [23]. Greater increase was observed in Group1 due to longer breath-hold durations, thus greater increase in cerebral blood flow occurred in Group1, pointing out to a better adaptation of the cerebral regulation in breath-hold divers.

Breath-hold indices for change in hemoglobin concentration and systemic oxygen saturation are calculated to obtain values normalized with respect to time in order to compare results of different groups with very different breath-hold times [33]. Both indices show significant difference between groups especially for the third and fourth breath-holds (statistical results can be seen in **Table 4.3** and **Table 4.5**. The appearance of statistical significance for the later breath-holds shows that difference between groups becomes more pronounced as the task becomes more difficult.

Greater desaturation in systemic oxygen saturation is expected in breath-hold divers at the end of breath-holds [8]. Systemic oxygen saturation remained almost constant in the control group over consecutive breath holds despite increase in breath-hold times. This may be a sign for a lack of short term adaptation. The increasing trend we observe in Group2 and the greater increase in Group1 means more effective diving response since body gets less and less oxygen as the breath-hold duration increases, in other words oxygen is conserved for vital organs.

Bradycardia was among the expectations of our study. A continuously decreasing heart rate is observed in diving animals but in humans we expected a less perfect bradycardia [14]. Although marked with abrupt increases continuous bradycardia is reported in some studies [6]. But these studies were task oriented constant time submaximal breath-holds. Continuous bradycardia during a maximal breath-hold can be a sign of very advanced adaptation. In very few subjects we observed bradycardia almost for the whole duration of the breath-hold. Average bradycardia durations increased in both diver groups but it increased more in experienced subjects and remained almost constant in the control group. Bradycardia duration percentages clearly show a better diving response in experienced breath-hold divers since on average their bradycardia lasted for 80% of the breath-hold duration while controls were able to sustain bradycardia for 50% of the breath-hold time. Group2, the group of beginner breath-hold divers stands between other two groups clearly showing the effect of breath-hold training, thus the adaptation can be learned and improved with continuous training.

Time parameters of the fits to breath-hold and rest signals reflected similarities between groups. Recovery times of [Hb] signal and systemic oxygen saturation are also similar. These similarities suggest normal cerebral and peripheral perfusion properties for all the groups [21]. This finding also supports that the systemic circulation has less variability among groups but it is the brain that was actually learned to adapt to breath-hold. On the other hand delay in the systemic oxygen saturation recovery may be a sign of lowered perfusion to less vital organs in the body which in turn may be accounted for peripheral vasoconstriction.

6. CONCLUSION

Three groups of subjects performed four consecutive maximal breath-holds for this study. Effects of maximal breath-holds along with the effects of consecutive breath-holds were investigated, similarities and differences between groups were analyzed.

We can clearly see an increase in breath hold durations for all of the three groups in **Figure 4.1**. If we assume that each subject terminated the breath hold approximately at the same personal level of discomfort then we can assume that the increase in breath hold durations is related to the short term adaptation to the task. When we further investigate the increases, we can see that the rate of increase is greater in Group2 (57%) than Group3 (46%) and even greater in Group1 (75%). This difference in increase rates can be associated with the adaptations to hypercapnic and hypoxic conditions of free divers.

Change in [Hb] is the main cerebral indicator for the level of breath hold. As the breath-hold continues there is a continuous increase in [Hb]. For longer breath-holds there is a greater increase. The amplitude of this increase shows the degree of adaptation of the subject since termination of breath-hold at an earlier time means a smaller change in [Hb] which in turn means a lesser tolerance to the resulting conditions of breath-hold task.

Change in systemic oxygen saturation follows a similar trend as the change in [Hb] signal and it reflects the response of the rest of the body. A greater decrease in saturation means less oxygen delivery to the rest of the body for conserving the oxygen for most vital organs, the heart and the brain.

Breath-hold indices reflect slope of overall change during breath-holds and it appears to be a successful index for differentiation between different groups. In the control group breath-hold indices remain almost the same for four breath holds. Since

subjects in Group2 and Group1 were able to extend their breath hold times we can see the increase in their performance as the increase in the breath-hold indices. A greater increase in Group 1 shows that the performance is related to experience of the subject.

We observed bradycardia during breath holds. Bradycardia is expected as a natural result of breath hold. But as subjects started to feel uncomfortable their heart rate increased again. Continuous decrease in the heart rate did not occur except some of the most experienced subjects. Experienced breath-hold divers were most successful in maintaining bradycardia during breath-holds.

When we look at systemic Oxygen saturations although some subjects approached the limit we can clearly see that none of the groups experienced a severe hypoxia. We can conclude that the termination of breath-hold is more likely related with hypercapnia. We can also claim that experienced divers are more resistant to hypoxia and hypercapnia since they were able to hold their breath up to a level that neither the control group nor the beginner divers can sustain.

7.   LIMITATIONS OF THE STUDY

During rest periods breathing rate of the subjects were not controlled. A constant breathing rate during rests could possibly reduce variations because breathing has instant effects on heart rate and as a result, on blood circulation.

Most of the subjects hyperventilated after breath-holds, altering their heart rate and possibly affecting our results, especially at the beginning of rests just after breath-holds. But it is very hard for the subject to control breathing rate after a maximum breath-hold and the difficulty increases as the breath-hold duration and the effort increases.

We intended to control inhales with BIOPAC Respiratory Effort Transducer but the device was unable to differentiate diaphragm contractions or any other involuntary movements from inhale maneuvers. Using the Effort Transducer would complicate the task without any additional information, so we decided to observe subjects for inhales to make sure that they held their breaths.

Some degree of uncomfortability is marked to be the trigger for the termination of breath-holds. But the subjectivity of the uncomfortability is the main problem for the comparison of different subjects. Experienced subjects know their abilities and their limits better, so they probably endured longer as they started to feel uncomfortable. We had to assume that subjects with similar experience levels have approximately the same threshold for uncomfortability caused by the breath-hold and the endurance comes as a result of training and adaptations.

8. FUTURE WORK

In our study we compared end results of breath-hold divers and controls for maximal dry breath-holds to observe the differences and limits of different groups. A study suggests smaller or even absent responses for breath-hold divers at breath-hold times, maximal for the control group [34]. Although greater desaturation in systemic oxygen saturation in breath-hold divers at the end of breath-holds are expected and obtained, a previous study found smaller desaturation in breath-hold divers compared to normal controls at earlier times during the breath-hold [8]. With the data at hand we can be able to perform these analyses by looking at the signals at chosen times.

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