Heart Rate Variability Analysis as a Means of Real-time Hypercapnia Detection

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Heart Rate Variability Analysis as a Means of Real-time Hypercapnia Detection

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy
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DEDICATION

I dedicate this manuscript to those brave men and woman who protect our freedom daily in hopes that its content will inform and enable their sustained superior performance.
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ABSTRACT

The motivation for this research is the high prevalence experienced by divers of carbon dioxide (CO₂) issues and concerns throughout their lives. Additionally, CO₂ is naturally occurring, the product of oxidative metabolism, and is undetectable to human senses. Current CO₂ sensor or detection technology is limited to high-cost machines such as arterial blood gas monitors and end tidal CO₂ monitors, or more modern technology relying upon bright lights which measure blood flow. The light technology is notoriously unreliable and does not produce a very effective heart beat allowing a user to detect minimal changes. Heart Rate Variability (HRV) is the change (or variability) in the timing between each heartbeat. There are few previous studies on this subject, but new research is being done on HRV now, and professional athletes are training using HRV as a performance and health indicator. Initial indications show that HRV may prove quite useful in detecting stress on the autonomic nervous system which is affected by CO₂.

The experimental methods we used were the following. The study population included 15 males between the ages of 18 and 50 years old and excluded those with pulmonary concerns, as identified by a physician, because of HRV confounding factors. This University of South Florida IRB approved study had minimal risk and noted numerous off ramps or stopping points to ensure subject safety. Each subject was normalized with respect to the known drivers for HRV and then tested breathing four
different breathing mixtures. Subjects breathed air as well as air containing 4\% CO\(_2\), 5\% CO\(_2\) and 6\% CO\(_2\) with air breaks in between each CO\(_2\) exposure to ensure no buildup of CO\(_2\). After being informed by the initial results of this clinical research study, a stand-alone device with an HRV sensing mechanism using a 3 lead ECG and proprietary software designed by the authors to manipulate the data collected and present it in a novel methodology was developed at the system level. The ultimate goal was to detect CO\(_2\) prior to the onset of hypercapnic symptoms. This novel system, including the analysis algorithm, relies upon comparing successive heart rates and plotting them in an ever-shifting time domain axis which creates a return plot map. Formulating an ellipse encompassing the majority of the data and detecting a change in eccentricity of that ellipse when a subject is breathing a different gas is the novel nature of the system.

Experimental results can be summarized as follows. The null hypothesis was that the mean of the ratio of the standard deviation of the long axis of the ellipse as compared to the standard deviation of the short axis of the ellipse would be the same for both the air breathing data set and the worst case of the CO\(_2\) breathing data set. A standard t score with a one tail t test was calculated. The t-value is 13.91. On a student’s T distribution table with a 99.95\% confidence interval and 28 degrees of freedom, the t-value would have to be greater than 3.6 to be significant. The p-value is < 0.00001. The null hypothesis was rejected. The results of the study were statistically very significant. The study clearly demonstrates a dramatic change in the plot given an identical time period for air breathing and CO\(_2\) rich air being breathed. The minimum change (or difference) in the ratio when breathing CO\(_2\) to breathing air was a difference
of 1.32 which represents a 34% change in ratio. The mean change was 1.93 (48.4%) with a 0.30 (5.9%) standard deviation.

In conclusion, while there are some known concerns and issues to overcome, the results are very promising for this pioneering study. This initial cohort study demonstrates that HRV monitoring is reproducible in our study group, did show a statistical change in response to elevated inspired CO₂, and correlates with CO₂ monitoring by other means. This type of HRV monitoring can be easily added via software upgrades to current ECG monitoring machines or manufactured as a stand-alone ECG/HRV monitoring device. This algorithm reduces the need for additional, more expensive CO₂ monitoring devices and arterial blood gas analysis. We further propose that HRV analysis can potentially provide an early warning system to detect hypercapnia related symptomatology prior to escalation of symptoms to a detrimental level and perhaps damaging or irreversible state.
CHAPTER 1: INTRODUCTION

Carbon dioxide (CO₂) is a colorless and odorless gas; although at very high concentrations it can have a sharp or acidic odor. At standard temperatures and pressures, the density of carbon dioxide is around 1.98 kg/m³, which is about 1.67 times that of air. Under most normal conditions CO₂ is undetectable to humans from visual, olfactory and glossopharyngeal standpoints. CO₂ is the byproduct of oxygen (O₂) metabolism in vertebrates, and present in higher concentrations in many naturally existing areas on earth such as volcanos, hot springs and geysers. Given the hazardous nature of the gas in high concentrations, and the fact that humans actively produce it, special consideration should be given for the ability to detect CO₂ prior to the onset of harmful physiological symptoms.

Heart rate variability (HRV) has been put forth by many including McCraty and Shaffer, of the Center for Applied Psychophysiology, at Truman State University as an indicator of cardiovascular health [1]. A basic definition of HRV is the change (or time-based variation) in frequency intervals between adjacent heartbeats. The QRS complex is a visual depiction of the human heart beat seen on a typical electrocardiogram (ECG, EKG). The QRS complex corresponds to the depolarization of the ventricles (both right and left) of the human heart and contraction of the ventricular muscles. These electrophysiological waveform deflections are visually shown as spikes which appear in rapid succession and reflect a cardiac cycle. A Q wave is a downward deflection immediately following the P wave. R waves follow the Q as an upward deflection, and
the S wave is a downward deflection after the R wave. For the purposes of timing a cardiac cycle, the peak-to-peak interval, or heart rate, is commonly measured from one R peak to the next R. HRV has been known to be related to the health of some of the body’s regulatory systems. For example, sympathetic and parasympathetic systems affect chronic variations seen in HRV. Specifically, McCratty and Shaffer emphasized that, "An optimal level of HRV within an organism reflects healthy function and an inherent self-regulatory capacity, adaptability, or resilience [2]." Generally speaking, the greater the HRV the better the health of the individual and less physiological stress. Decreasing HRV indicates a possible pathology or poor function in levels of the self-regulatory control systems and could predict numerous risk factors [3].

The goal of this dissertation is to design and test a system that can monitor the QRS complex and discern frequency variability or HRV by detecting the R and the R+1 (or successive R) temporal intervals at high temporal resolution. The generalized dissertation rationale is that an acute metabolic condition related to elevated CO₂ serum levels will induce a decrease in heart rate variability thereby making this condition detectable prior to symptoms being felt by the patient, or causing any type of permanent physiological or anatomical damage.

This system will have sufficient power to operate for periods up to 24 hours and collect the required data for real-time display as well as data analysis. We hypothesize that HRV can be used to analyze and detect changes in physiological stress conditions, and that it will be useful for detection of hypercapnic states in a variety of medical and healthcare environments. The connected background calculations will feature a Poincaré plot or “return map plot”. These plots can be used to distinguish physiological
stress from background noise or chaos by embedding a data set into a higher-dimensional state space. The plots will feature a time series of the form $R$ at any time $(t) = R_t$ and the next successive $R = R_{t+1}$. This return map will plot $(R_t, R_{t+1})$ first, then it plots $(R_{t+1}, R_{t+2})$, then $(R_{t+2}, R_{t+3})$, and so on. This tool can accurately detect variability between two sets of time domain data consecutively by using a correlation analysis. Of course, a higher correlation coefficient indicates decreased HRV, an indication of dysfunctional self-regulatory control systems. The intellectual merit of this activity is significant because this is the first-ever system to alert a user to indicate HRV pathology or poor function, for various levels of self-regulatory control aspects of the cardiovascular system.

1.1 Motivation for Research

This dissertation project’s prime motivation was to develop an autonomous simplified system to determine shifts in HRV which are trending towards decreasing variation, which indicate a pathology or poor function in various levels of self-regulatory control systems just prior to subject discomfort, and the onset of signs or symptoms of hypercapnia. As stated above, CO$_2$ is a colorless and odorless and a problem when in enclosed spaces. Additionally, while serving on active duty in the U.S. Navy, the author experienced hypercapnia while diving a closed-circuit oxygen rebreather and at least one person was reported as having succumb to hypercapnia while diving.

Symptoms and signs of early hypercapnia include flushed skin, increased pulse pressure, tachypnea, dyspnea, premature contraction of the heart, myoclonic twitches, asterixis, headache, confusion and lethargy. In cases of severe hypercapnia,
symptomatology progresses to disorientation, panic, hyperventilation, convulsions, unconsciousness, and possibly death.

In common medical practice, two such modalities are utilized, end tidal CO\textsubscript{2} (EtCO\textsubscript{2}) measurement during respiration, and arterial blood gas (ABG) sampling. These modalities monitor serum CO\textsubscript{2} through direct or indirect methods; however, they do not identify impending pathological responses to the hypercapnia. Humans respond differently to hypercapnia and as such some individuals are able to withstand higher levels of CO\textsubscript{2} without significant consequence. Our critical assessment of previous research led to our hypothesis that HRV is a monitorable physiological biomarker that can predict the onset of pathological hypercapnia, which is not measurable by the current standard-of-care methods of EtCO\textsubscript{2} and ABG.

For Self-Contained Underwater Breathing Apparatus (SCUBA) divers, CO\textsubscript{2} buildup has been a known, serious, recurring and long-term cause of maladies. Hypercapnia in SCUBA divers can lead to simple symptoms such as headaches or cause profound symptoms such as loss of consciousness or drowning and death. In diving, CO\textsubscript{2} exacerbates the symptoms of Nitrogen Narcosis and is known to have more severe narcotic effects than nitrogen alone due to the lipid solubility of CO\textsubscript{2}. So, in open circuit SCUBA diving, monitoring of CO\textsubscript{2} is important with respect to narcosis. In rebreathers, CO\textsubscript{2} must be removed from the breathing system, usually by a scrubber containing a solid chemical compound with a high affinity for CO\textsubscript{2}, such as soda lime. If not removed from the system, CO\textsubscript{2} may be re-inhaled, causing an undesirable increase in the inspired CO\textsubscript{2} concentration. Failure, channeling or bypass of the scrubber
Some diving system designs force a “dead space” which does not allow all of the CO$_2$ to escape to the environment. An example of this would be an elongated snorkel device which will extend out of the water but never be fully evacuated because of its design length. Another way a diver can experience an increase in CO$_2$ is over-breathing or over-exercising. Over-exercising produces excess CO$_2$ due to elevated metabolic activity, often in the face of voluntary hypoventilation to preserve breathing gas, thereby rapidly increasing CO$_2$ inspired concentrations. The density of all breathing gas is higher at lower depths, so the effort required to fully inhale and exhale is increased, making breathing more difficult and less efficient. The higher gas density also causes gas mixing within the lung to be less efficient, thus increasing the dead space. Even with normal respiration, SCUBA divers may experience alveolar hypoventilation resulting in inadequate CO$_2$ elimination or hypercapnia. Additionally, rapid descent rates by divers can have similar impacts because the divers are unable to effectively remove CO$_2$ from their lungs due to rapidly increasing pressure on the lungs and incomplete exhaust from the lungs. Finally, and perhaps most importantly, CO$_2$ buildup hastens the onset of CNS O$_2$ toxicity and the synergistic effects of the two gasses in combination is significantly more damaging than either gas alone. [4]

1.2 Limitations of Current CO$_2$ Detection Technology

Current medical CO$_2$ sensors range from $1,000 to $3,000 and up. Mass spectrometers are much costlier and require considerable calibration and maintenance to run effectively. Most medical CO$_2$ detectors in hospitals or clinics rely upon costly
disposable sensors and must sample the patient’s expired breath in order to be effective. These sensors measure end-tidal CO\(_2\) in expired air, which unfortunately does not discern if the level of CO\(_2\) is problematic within a particular patient or diver in real-time; so not in line with current movements towards personalized medicine in health care. A low-cost and effective alternative is needed to be able to identify hypercapnia early and quickly prior to the onset of CO\(_2\) related symptoms within patients or divers.

Some diving equipment manufacturers have introduced CO\(_2\) sensors, but they suffer from the same problems as medical sensors because they can only determine inspired CO\(_2\) amounts. This is only partially helpful as CO\(_2\) affects people at different thresholds. Alternatively, manufacturers have developed temperature sensing devices within the CO\(_2\) scrubbing materials which can determine if the exothermic reaction is propagating toward the end of life of the material, giving the user a possible indication of CO\(_2\) breakthrough (or a predictor of hypercapnia), however this type of a sensor does not indicate if CO\(_2\) is problematic for a particular person in real-time.

Some manufacturers have attempted to use a laser or infrared sensors and a sensing pad to analyze CO\(_2\) for inhaled and exhaled breaths. These nondispersive infrared sensors rely on the optical path to measure the concentration of CO\(_2\). Water vapor can condense on the optical elements of the sensors blocking the signals the sensors use, resulting in faulty CO\(_2\) readings. In sum, no portable, cost-effective device or system exists today which can provide accurate early detection of hypercapnia as it becomes problematic in a patient.
1.3 Previous Studies on this Subject

A literature review suggests that HRV has been described by many as an indicator of cardiovascular health [3]. HRV is the change or temporal variation in frequency intervals between adjacent heartbeats. Sympathetic and parasympathetic systems have been shown to affect these temporal variations [5].

An optimal level of HRV within an organism reflects healthy function and an inherent self-regulatory capacity, adaptability, or resilience. Generally speaking greater HRV is an indicator of better health and less physiological stress [3]. Decreasing variation indicates a pathology or poor function in levels of self-regulatory control systems [5,6].

Previous reports have demonstrated the power of HRV as a predictor of chronic diseases such as COPD [7] as well as its insights into adequacy of autonomic compensation to severe trauma [8]. The present study was performed to evaluate if HRV will decrease in response to acute hypercapnia and develop and test a novel device to provide early detection of carbon dioxide (CO₂) related symptoms before the onset of detrimental or permanent health effects in humans.

There are very few studies evaluating HRV and its clinical applications [9]. HRV analysis has been used to monitor fetal distress in conjunction with Neonatal Critical Care, as well as the effects of exercise training on HRV in patients with hypertension. There are a number of reports of HRV analysis during surgeries and related to several other chronic medical/surgical conditions [9]. None of these studies have focused on the biomedical engineering aspects of developing and manufacturing a purpose-built HRV monitoring and alert system for early detection of dangerous levels CO₂. While there are
myriad scholarly articles in exercise physiology, few if any relate to CO₂ and HRV real-time analysis and detection systems.

Finally, Pöyhönen and colleagues added CO₂ to inspired gas and showed that this increased High Frequency (HF) and Low Frequency (LF) components of HRV in normal ventilation conditions. [10] Breathing frequency is known to alter HRV independent of partial pressure of CO₂ (PaCO₂). They further went on to discuss the effect of PaCO₂ appears to be related to levels of consciousness. This suggested a modulation of the autonomic nervous system (ANS) activity would contribute to the effects of PaCO₂ on HRV [10] showing some promise as an example of research which has in part inspired the present dissertation project.

The fact that attempts at conscious control of breathing frequency had no significant effect on HRV [11], and that nonlinear HRV analysis using short term ECG can be effective to detect real-life stress condition [12] makes HRV a perfect determinate for ANS health because HRV cannot be consciously influenced by the subject.

Poincaré Plots have been used in the past and in some ways shown a relationship among LF, HF, length, and width [13] of the plot. However, no current literature indicates that researchers are using the Poincaré Plots to detect acute stress by using an ellipse fitting algorithm, and comparing a ratio of the standard deviation of the short axis to the long axis.

1.4 HRV Background

HRV has been predicted and interpreted by using ECG waveforms. Generally speaking the ECG test records the electrical activity of a human heart through small
electrode patches that are attached to the skin of the arms, legs and chest. ECGs are quick, safe, and painless and often used as a diagnostic test for checking heart rhythm, see if there is poor blood flow to your heart muscles (called ischemia), to diagnose a heart attack and to check on things that are abnormal, such as thickened heart muscle. In this case the ECG is very reliable for determining the QRS complex and accurately depicting the exact timing of each successive R peaks as pictured in Figure 1.1. For the purposes of this project the amplitude of the R peak was ignored and only the timing was used. The R was chosen because it is the steepest / sharpest change in amplitude and therefore the easiest to detect.

Figure 1.1: Standard 12 Lead ECG
The objective was to develop a device and an algorithm that can detect a shift in HRV and use it as a predictor of a change in patient health prior to detectable symptoms or permanent problems. Since HRV is a predictor of physiological changes in the human body, we believe continually monitoring HRV could forewarn users of impending physiological issues signifying a change in overall physiological health.

As previously discussed, HRV is the change or temporal variation in frequency intervals between adjacent heartbeats as pictured in figure 1.2. The HRV measurement was accomplished by analysis of the standard electrocardiography QRS complex tracing. Detecting the time between the two successive R peaks (R_t, R_{t+1}) and the time between the next two R peaks (R_{t+1}, R_{t+2}). This HRV measurement algorithm was used here-in to analyze and detect changes in potential physiological stress conditions such as hypercapnic states. This was accomplished using a modified Poincaré plot or “return map plot”.

![Figure 1.2: Drawing of a Waveform Representation of Temporal Variance of Frequency Intervals Between Adjacent Heartbeats.](image)

This return map plots as coordinates (R_t, R_{t+1}) then (R_{t+1}, R_{t+2}), then (R_{t+2}, R_{t+3}), and so on. This analytical tool can be used to accurately detect variability between two consecutive time domains using a correlation analysis. Higher correlation coefficients indicate less variability and are a sign of dysfunctional self-regulatory control systems.
[4]. As shown in Figure 1.3, the line representing the standard deviation of the short axis (SD1) and the line representing the standard deviation of the long axis (SD2) are in a ratio of approximately 4:1 on the left side, which represents a normal R-R ellipse. In a distressed R-R ellipse, pictured on the right of Figure 1.3, the ratio can be as little as 1:1. These plots along with an ellipse fitting algorithm were used to distinguish stress from chaos by embedding a data set into a higher-dimensional state space. The change in eccentricity of the ellipse is how the algorithm was able to track and predict physiological distress.

Figure 1.3: Drawings of Poincaré Plots with Pictorial Representation of the Ellipse Fit Algorithm with Short-axis Standard Deviation (SD1) and Long-axis Standard Deviation (SD2) Marked on Both a Normal R-R Ellipse (left) and an Assumed Representation of a Distressed R-R Ellipse (right). See Text for Further Axis Unit Definitions.
CHAPTER 2: EXPERIMENTAL DESIGN

The study’s purpose was to determine if shifts in HRV which are trending towards decreasing variation (potentially indicative of pathology or poor function in various levels of self-regulatory control systems) would appear prior to subject discomfort and the onset of more severe signs or symptoms of hypercapnia. The crossover design study helped to answer the research question, “Can HRV be used to predict hypercapnia prior to physical symptoms or accepted clinical signs of hypercapnia?”.

The N of 15 human subjects was selected as a proposed N for purposes of collecting initial, preliminary data for proof-of-concept. Since this type of study has not been completed before, we have no similar previous data upon which to perform a power analysis. These initial data will provide the necessary information to provide a power analysis for follow-up investigations.

2.1 Study Population Inclusion and Exclusion Criteria

Inclusions were male subjects 21-50-years (a normally distributed group, N=15) were recruited by the International Board of Undersea Medicine for this study by Joseph Dituri per USF IRB guidelines for acceptable and recruitment methods. Exclusions were all female subjects; subjects with respiratory health problems as measured by vital capacity; subjects with any physiological conditions or abnormalities; as well as any condition the supervising physician excludes;
Variations in female hormone levels associated with menstrual cycles in this age group have a possibility of impacting HRV values, therefore females were excluded from this initial study. On the physical exam, if a potential subject breathing insufficiency was detected, i.e., insufficient vital capacity, the subject was excluded. Also, major health problems involving the respiratory system would have been excluded from this initial study. No subjects from the proposed group met the exclusion criteria therefore none were excluded.

2.2 Potential Study Risks

This study was approved by the Institutional Review Board (IRB) at the University of South Florida [CR1_Pro00030609] as shown in Appendix F. Minimal risk was associated with the sticking pad, which may cause irritation for the ECG electrodes. Additionally, fasting may cause dizziness or lightheadedness. Mild hypercapnia symptoms can include headache, confusion and lethargy. Hypercapnia can induce increased cardiac output, which will decrease HRV as well as induce an elevation in arterial blood pressure and increase in arrhythmias. Symptoms and signs of early hypercapnia include flushed skin, full pulse, tachypnea, dyspnea, premature contraction of the heart that is independent of the normal rhythm of the heart, muscle twitches, hand flaps, reduced or abnormal neural activity, and oftentimes a raised blood pressure. In severe hypercapnia symptomatology progresses to disorientation, panic, hyperventilation, convulsions, and unconsciousness. Hypercapnia is generally caused by hypoventilation, lung disease, or diminished consciousness, but as described later could be hastened by CO₂ scrubbing system failures, rapid diving descents and increased partial pressure of gasses.
A medical doctor with extensive experience in hyperbaric conditions, USF Assistant Professor Dr. Farhan Siddiqi, was present during all testing and a crash cart was readily available, along with O\textsubscript{2} along with necessary resuscitation equipment was in place in the unlikely event it was required. Dr. Siddiqi was trained in the use of these devices and had the capacity to affect a rescue or resuscitation, but it was not required. All patients were monitored continually for flushed skin, dizziness, rapid breathing, increased blood pressure, increased heart rate as well as muscle twitches and any other potential malady which could be attributed to hypercapnia.

2.3 Off Ramps or Stopping Points to Ensure Subject Safety

If there was a recognizable shift in skin color, reported dizziness, 20% increase in respirations or breathing pattern, 20% increase in blood pressure, 20% increase in heart rate or any visible or reported muscle twitches, the subject would have been switched to air regardless where they were in the protocol and observed until their end tidal CO\textsubscript{2} goes below 40 torr. The physician would then perform a full physical examination immediately to ensure the health of those individuals with any symptoms. If an off ramp was observed, this subject would have been terminated and results noted. The participants were all asked for to provide a “GO” or “NO GO” response every other minute and the results were recorded. Each subject was instructed to end the experiment at any time should they not feel comfortable by giving the NO GO sign. An additional "off ramp" shall be if a change in HRV is detected specifically a greater than 50% change in the ratio of mirrored access in relation to the short access of a Poincaré plot (normally a ~3:1 difference). An example would be a 50% change from ~4:1 to ~2:1.
Additionally, if greater than 3 subjects experience an “off ramp” or “stopping point” event, the study would have been stopped for a potential risk vs reward evaluation.

Data collection was performed at the International Board of Undersea Medicine (IBUM) building at 701 N. Westshore Blvd., Tampa, FL 33609, using a standard 12-lead ECG for the purpose of detection of HRV anomalies with varying levels of CO₂ breathed by individuals and to determine if HRV can predict carbon dioxide increases prior to hypercapnia symptom onset.

A series of gases were purchased from Air Liquide with verified contents of 6% 5% and 4% CO₂. Each contained 21% O₂ and the remaining portion (balance) was nitrogen. These three separate gas amounts were cascaded into individual SCUBA cylinders containing the same contents in smaller, more maneuverable cylinders. Each cylinder contained between 2000 Pounds Per Square Inch (PSI) and 3000 PSI. A fourth SCUBA cylinder containing only air (21% O₂ and 79% nitrogen) from a grade E certified pure compressor was used.

The four SCUBA cylinders were connected to four different Hollis and Poseidon Deutsches Institut für Normung (DIN) first stage regulators. The gas pressure was reduced from between 2000 PSI and 3000 PSI to approximately 135 PSI. The low pressure lines from each individual regulator were cascaded into a common manifold. A single second stage regulator was connected as an outfall from the common manifold and routed to the test subject’s mouth. Each SCUBA cylinder was carefully labeled with content decals and properly ordered such that no mistakes were made during the administration of gas to the subjects.
The exhaust from the second stage regulator was directed to a half inch hose which was connected to a Smiths Medical Capnograph (Model 8400) for sampling of end-tidal CO₂. The exhaust was further directed into the intake of the air conditioning system in the facility to direct and distribute the carbon dioxide laden exhaust away from the subject and staff performing the research. The subject’s pulse oximetry / percent O₂ was also monitored via a transcutaneous finger lead.

The subject was fitted with a Nasiff cardio resting (Model CUSB Rev A7) 12 lead electrocardiogram using Fastrace ECG leads as seen in figure 2.3 below. The subject’s entire cardiac cycles were monitored throughout the duration of the testing.

Figure 2.1: Visual Representation of a Study Subject.
The entirety of the test was videoed using a GoPro Hero 5. The subject's blood pressure was taken using a Longs Drugs model BP 3AA1-1 every 5 minutes and recorded on paper.

At least 48 hours prior to participation in the study, the participant underwent a physical examination including a neurological assessment prior to being allowed to participate in the study, and subjects with any physiological conditions or abnormalities, including cardiovascular problems were excluded by our Study physician.

The overall duration of a study for an individual was in all cases less than 5 hours total. All subjects were required to fast for 10 hours prior to ECG tests to minimize the impact of metabolic differences on the subject’s HRV. All subjects were in good physical health and were instructed not perform exercise for 10 hours prior to ECG tests.

Nicotine and caffeine were allowed during the study but not during the 10-hour fast prior to the study. All subjects kept a lifestyles diary during the study with food intake, nicotine intake, caffeine intake, drugs, sports or dietary supplements, alcohol intake and any medications taken for 48 hours prior to the study period. Additional entries were to be made in the diary concerning stresses experienced within 10 hours of the study. During the fasting process water was acceptable.

The subjects all went through an identical intake process consisting of notification of subject’s rights, discussion and signature of consent forms along with a relaxation period. At the intake the time consent was re-affirmed by the participant. Subjects entered into the testing room and laid upon the subject table in a dimly lit, silent room. The study coordinator connected the ECG leads to the subjects and connect them to the breathing apparatus which was set to deliver normal air.
A 20-minute acclimatization period was afforded to accommodate those not comfortable with the breathing device (if required) in order to standardize the protocol and allow for adaptation in comfort levels. During that time, a baseline HRV was established for each participant. Additionally the blood pressure and pulse was monitored every 10 minutes during study. Familiarization with the chest breathing apparatus device, as well as with environment where the tests were performed was critical since these factors can modulate HRV biomarkers. It was important to make sure that the subjects were as comfortable as possible with the experimental process prior to taking the HRV measurements.

Prior to measuring HRV, a 20-minute acclimatization period was observed with low ambient room lighting, similar room temperature and no audible or visual disturbances while connected to the ECG. The entire study was recorded with video and audio settings to monitor and record any behavioral symptoms of CO₂ toxicity. This condition remained consistent during all subsequent testing throughout the entire study. Individual test subjects served as their own control while they were exposed to air during the data collection cycle. There were three 10-minute exposures to high CO₂ breathing periods which are stepwise increased. Exposure levels of CO₂ were 4%, 5%, and 6%. The mixtures will be as seen in Table 2.1 below.

Each cycle started with a 20-minute acclimation period followed by a step wise increased exposure to CO₂ for 10 minutes followed by a 20 min breathing air (recovery period) or until ETCO₂ goes below 40 torr or complete CO₂ washout to ensure reversibility followed by another 10 min breathing of a predetermined mix to assess
post- CO₂ HRV. This continued for four cycles of breathing all the gasses listed in Table 2.1 in a stepwise increasing CO₂ exposure order.

Table 2.1: List of Gasses and Quantities

<table>
<thead>
<tr>
<th></th>
<th>Oxygen %</th>
<th>Nitrogen %</th>
<th>Carbon Dioxide %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mix 1</td>
<td>21</td>
<td>79</td>
<td>0</td>
</tr>
<tr>
<td>Mix 2</td>
<td>21</td>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td>Mix 3</td>
<td>21</td>
<td>74</td>
<td>5</td>
</tr>
<tr>
<td>Mix 4</td>
<td>21</td>
<td>73</td>
<td>6</td>
</tr>
</tbody>
</table>

2.4 Human Subjects Considerations

Description of the informed consent process: At least 48 hours before any experimental procedures subjects were interviewed and presented with all study materials and consent forms to review in detail with the Study Coordinator and physician. Upon arrival on day of study they re-affirmed their desire to consent.

Discussion of how the privacy and confidentiality of the subjects was maintained: The paper file for each subject is stored in a locked file cabinet. All computer data files are password protected.

This is viewed as a minimal risk study in that similar carbon dioxide percentages exist in the normal method for saving lives such as Cardio Pulmonary Resuscitation (CPR). We used 10-minute exposures. Lambertsen, (1971) established safe stay times in CO₂ and we are only slightly above one of those for shorter durations. [14] Lambertsen recommends a safe stay time of 6% for a period of 30 minutes, consistent with our use of 6% at 10 minutes to elicit a hypercapnia symptom and physiological response. The present study is low risk for three 10-minute exposures to 4%, 5% and
then 6% with adequate direct physician supervision and adequate "off-ramps" at the first indication of any signs of hypercapnia. Lambertsen states that still higher concentrations should be respirable for many hours without acute harm or residual effects.

2.5 Specific Contribution to the Field

This project will advance discovery and understanding while at the same time promoting learning by demonstrating a correlation between HRV and common stresses within the body such as hypercapnia. The results of the project will be disseminated broadly to enhance scientific and technological understanding. The potential benefits of this research, to society at large, are innumerable in that this physiological measuring system can be used external to the body with non-invasive technologies, yet can potentially detect and warn against a number of serious, internal physiological life-threatening conditions. Some conditions proposed along with hypercapnia are COPD, MDS/anemia, Decompressive Stress and Oxidative Stress.

The intellectual merit of this proposed activity is significant because this would be the first ever designed device to alert a user to indicate a pathology or poor function in various levels of self-regulatory neural control aspects of the cardiovascular system. A USF patent was filed for a system which is described below and meets the shortcomings and limitations identified above.

2.6 Device Design

The system which was used to collect the data for the human subject study took an analog signal from a commercially available ECG machine which ported out the R time interval for each successive heartbeat. This signal was fed via a 3-conductor 1/4" Phone Plug into an MSP430F5529 Texas Instruments LaunchPad (pictured in figure 2.2
below) which allowed the signal to be converted from analog to digital, and a program written in Python for ease of display and data analysis.

![Figure 2.2: MSP430F5529 Texas Instruments LaunchPad and Phone Plug](image)

Foremost for the final device design, the Python program in Appendix A needed to be converted to the C programming language. Initial application for a provisional patent was applied for during cultivation of the idea and manufacturing of the prototype. Following the one-year period for provisional patent, a utility patent has been applied for by the author and the University of South Florida Technology Transfer Office.

The purpose of this prototype system was to realize the python algorithm engineered by the author on an embedded platform, and to better understand the hardware implementation and requirements. For instance, the python implementation provides a file containing the times associated with the measured ECG R peaks.

For prototyping purposes, we used a commercial off-the-shelf single lead ECG (3 electrodes) monitor board. The monitoring board uses ANALOG DEVICES Single-Lead, Heart Rate Monitor Front End device, AD8232. The AD8232 is an integrated signal
conditioning block for ECG and other biopotential measurement applications. It is
designed to extract, amplify, and filter small biopotential signals in the presence of noisy
conditions, such as those created by motion, muscle movements or remote electrode
placement. This design allows for an ultralow power analog-to-digital converter (ADC) or
an embedded microcontroller to acquire the output signal easily. The AD8232 can
implement a two-pole high-pass filter for eliminating motion artifacts and the electrode
half-cell potential. This filter is tightly coupled with the instrumentation architecture of the
amplifier to allow both large gain and high-pass filtering in a single stage, thereby saving
space and cost. An uncommitted operational amplifier enables the AD8232 to create a
three-pole low-pass filter to remove additional noise. The user can select the frequency
cutoff of all filters to suit different types of applications.

To meet the 10 hours run time requirement for this device, a Lithium Ion Polymer
Battery 3.7V 2500mAh was chosen. With the entire Heart Monitoring box consuming
about 150mA, this battery should provide approximately 16 hours of run time
(2500mAh/150mA = 16h). There is a 500mA fast acting safety fuse installed to prevent
shocking risk. The battery charger selected is a USB Lilon/LiPoly Charger – v1.2. This
allows recharging via USB port and the fast-charge current is 500mA by default but is
easily adjustable from 100mA up to 1000mA. There are also separate JST connectors
for battery and load system, so the battery does not need to be removed during
charging.

The Heart Monitoring unit uses a Kinetis K66 microcontroller unit (MCU). The
MCU is a 32-bit 180 MHz Cortex-M4 processor. Due to the heavy calculations required
by this device, a normal 8-bit MCU would not suffice. Compared to an 8-bit processor,
this MCU will fetch and write four times faster because of its 32-bit architecture. This MCU also comes equipped with a floating-point unit (FPU, a math coprocessor), which is necessary to perform non-integer math operations. Another requirement of the MCU was a high-resolution ADC. The ADC is necessary to read the output signal of the AD8232 device. The MCU ADC has a 10-bit resolution which means the lowest voltage level that it can detect from the AD8232 is \( \frac{3.3}{2^{10}} = \frac{3.3}{1024} = 3.2 \text{mV} \). For ease of prototyping, a Teensy 3.6 MCU development board was used. The development board incorporates the Kinetis MCU. The development board also comes with 1 Megabyte Programmable Flash. This flash size provides adequate storage for the application firmware and supports future enhancements of the application firmware. Last, there is an onboard micro SD card port for datalogging along with a USB port for programming and debugging.

The Pan-Tompkins algorithm [15] was used for real-time detection of the QRS complexes of ECG signals. This algorithm reliably recognizes QRS complexes based upon digital analyses of slope, amplitude, and width. It has a digital bandpass filter to reduce false detections caused by the various types of noise present in normal ECG signals. The chain of filters run is a DC block, low pass @ 15 Hz and high pass @ 5Hz. The filtered signal goes both through a derivative filter, which output is then squared, and through a windowed integrator. This filtering permits use of low thresholds which increases the detection sensitivity. The algorithm adjusts thresholds and parameters as required to automatically adapt to ECG changes making it highly accurate for detecting QRS complexes.
The display is a 2.28” Thin-film-transistor liquid-crystal display (TFT LCD) with a Serial Peripheral Interface (SPI). This was selected because only 4 pins are required to communicate with the LCD and Arduino support makes for an easy code development environment.

The box housing the components was 3D printed with ABS filament. The unit has a pushbutton which is used to START and STOP a test. The unit also contains a haptic motor which alarms if the ratio of HRV changes >25%. The LED toggles with the detection of a QRS peak according to the Pan-Tompkins algorithm. The unit comes with a USB interface which makes it convenient and fast to transfer data from the MCU to a host PC for post processing and firmware updates.

The device had significant timing constraints which had to be allowed for and compromises made. The MCU samples the AD8232 output signal (ECG data) at 1 KHz, which equates to a period of 1 millisecond (ms). It takes 56ms to update the TFT LCD Screen and to store the latest datapoint to the SD Card. The time required to calculate the math for the ratio of SD1 : SD2 is about 40 ms. The 1ms sampling period is achieved with a timer callback function. When 1 ms has expired, a flag is set which allows the Pan-Tompkins algorithm to retrieve a new sample. The benefit using a timer-callback approach is that it does not block the execution of MCU and therefore it can continue to process the RR ratio while waiting for the next sample. However, during the LCD and SD Card update, the interrupts must be disabled to prevent interruptions in the communication stream between the MCU and LCD and SD-card. It is important to note that since the LCD and SD card update takes about 56 ms, 56 samples will be missed during update. Since most individuals have between 500-1500 ms RR interval, this is
not be an issue, however the LCD update must be programed to happen immediately after a peak sample has been detected.

Firmware was development using the Arduino Integrated Development Environment (IDE) version 1.8.6 and Teensyduino version 1.44. The following three functions (panTompkins, time_average_rr_ratio, and calculate_rr) are responsible for the realization of our new algorithm.
CHAPTER 3: EXPERIMENT RESULTS

The human subject data set for this initial investigation consisted of twelve Caucasian males, two Asian males and one black male. The age of subjects was a mean of 38.7 years old with a standard deviation of 9.7 years. A careful review of the daily food logs of each subject was conducted. Because this was an *ex post facto* representation of their diet and the subjects were only told to record what was consumed, there is little similarity between subject’s caloric intake or diet, and no evidence of any correlation between diet and HRV changes were observed.

The data was recorded on a 12 lead off-the-shelf ECG. This ECG has a real-time port which sends the R signal from the QRS complex out repeatedly. The new program was written in C language and detailed in Appendix A took this real-time input and processed it for visual representation of the data by plotting the R as (X,Y) coordinates using the procedure of first plotting \( (R_t, R_{t+1}) \) then plotting \( (R_{t+1}, R_{t+2}) \), then \( (R_{t+2}, R_{t+3}) \), and so on where \( t=\text{time} \). The program in Appendix A afforded the research team the ability to view the changes in HRV, associated with breathing different gasses, in real time. This analytical tool was used to accurately detect variability between two consecutive time domains using a correlation analysis. The program then computed the SD1 and SD2 of the produced ellipse and compared them to each other in a ratio. The program then represented the 1 minute, 5 minute and full duration average of the ratio.
for comparison of changes. As the ratio reduced, the visual representation demonstrated the change of the HRV.

One hundred percent of the subjects displayed a detectable change in HRV. None of the subjects showed any clinical symptoms of hypercapnia nor did any of them request to terminate or were terminated for medical concerns by the attending physician. Observations for confounding variables that could affect the HRV was performed and there was no recognizable shift in skin color, dizziness change in respiratory rate, blood pressure, or heart rate of subjects of greater than 20% in any subject, cue to changes in HRV.

However, the changes in HRV associated with changing breathing gasses, instead of shrinking and coalescing into a smaller circle as pictured on the right side of figure 2.2 above, as we hypothesized initially, tended to expand the variability spread into a larger circle. The SD of the short and long axis did converge and approach a 2-1 ratio or less, but instead of reducing the size of the plot, the size was increased. SD1 tended to increase its length as opposed to SD2 reducing its length. The net result is a change in the ratio but not in a manner that was initially expected.

The null hypothesis was that the “mean of the ratio of the SD2:SD1 would be the same for both the air breathing data set and the worst case of the CO₂ breathing data set.” The mean difference of the air breathing set (X-Mean= M1) was 3.99 while the mean difference from the CO₂ breathing data set MSD2:SD1 (Y-Mean= M2) was 2.06 while the square of the mean of air breathing set ((X-Mean)² = SS1) was 2.61 and the square of the mean of the ((Y-Mean)² = SS2) was 1.44.
A standard t score with a one tail t test was calculated. The t-value was 13.91.

The p-value was < 0.00001. The null hypothesis was rejected.

![Figure 3.1: Demonstration of Changes in HRV When Breathing Air and CO₂. Subject side-by-side comparison HRV plot (left) on air and (right) after introduction of higher concentration CO₂. Both from subject on Feb 28th 2018.](image)

A visual comparison (Figure 3.1 from our clinical research study) clearly demonstrates a dramatic change in the plot given an identical time period picture for both left (air) and right (CO₂). A quantitative comparison shows a decrease from a 4.10 : 1 ratio to a 2.31 : 1 ratio of long axis to short axis. The results for Figures 3.2 and 3.3, for two more representative subjects, are similar, starting from a subject's baseline (air breathing portion) on the left and a section of increased CO₂ breathing on the right.

The full representation of the data is provided in Table 3.1 below. The minimum change (or difference) in the ratio when breathing CO₂ to breathing air was a difference of 1.32 which represents a 34% change in ratio. The mean change was 1.93 (48.4%) with a 0.30 (5.9%) standard deviation.
Table 3.1: Full Data Showing Long and Short Axis of Ellipse Comparison and Ratios as Well as Age of Subjects.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Air ratio</th>
<th>CO2 Ratio</th>
<th>Difference</th>
<th>% change</th>
<th>Diff^2</th>
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<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>4.09</td>
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<td>2.22</td>
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<td>2.04</td>
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<td>0.32</td>
<td>0.30</td>
<td>5.90</td>
<td>1.15</td>
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</table>

Figure 3.2: Demonstration of Changes in HRV When Breathing Air and CO₂. Subject side-by-side comparison HRV plot (left) on air and (right) after introduction of higher concentration CO₂. Both from subject on March 2ⁿᵈ 2018.
Figure 3.3: Demonstration of Changes in HRV When Breathing Air and CO₂. Subject side-by-side comparison HRV plot (left) on air and (right) after introduction of higher concentration CO₂. Both from subject on March 8th 2018.
CHAPTER 4: DISCUSSION

It appears there is a clear difference in the Air and CO₂ breathing conditions, as well as for the quantitative, graphical representation of the data while breathing CO₂. In each case, the plots returned to near baseline once the subjects were removed from the gasses rich in CO₂ and the EtCO₂ reduced below 40 torr. We have demonstrated that a change in HRV is not only detectable but would be a good tool to display the stress-related to breathing CO₂ prior to the onset of significant clinical hypercapnic symptoms. While the data are not as clear as our initial hypothesis purported, there is clearly a discernable change in HRV using the Poincaré plot analysis and the modified ellipse fitting algorithm by comparing the ratio of SD2:SD1 over multiple temporal iterations.

The data were analyzed in different ways and the ultimate useful data collection was achieved iteratively. The most successful combination was to compare the 1-minute, 5-minute and 10-minute ratio of SD2:SD1 for all data. Initial data collection seemed to indicate an ANS response to a stress (in this case increased levels of CO₂) to occur within 2 minutes of switching gasses; so, suggestive of a response time of less than 2 minutes. The running 1-minute, 5-minute and 10-minute ratio each continued and moved in concordance. Specifically, the 1-minute ratio was the first part of the 5-minute and 10-minute ratios as was the 5-minute ratio the first part of the 10-minute ratio, and all ratios moved together in a first-in first-out methodology with respect to time. This allowed the data analysis to capture the maximal change in ratio, as well as span the entire gas change period which was limited to 10 minutes/CO₂ concentration.
Multiple groups of data were collected on each subject, including vital signs, EtCO₂, pulse oximetry, ECG and HRV as well as dietary and stress information from the previous 48 hours via questionnaire. Vital signs including respirations, pulse and blood pressure did not change more than 20% for any of the subjects, and often much less than 20%. The reasoning for not investigating further into devices or systems which could monitor vital signs was that type of monitoring usually impacts the subjects by increasing their blood pressure due to the extra monitoring equipment applied to them. Pulse oximetry had a minimal change of 2% overall and did not seem to correlate with gas changes, making it ineffective for prediction of CO₂ levels.

A review of the diet consumed did not reveal and contributing factors, as expected. Since the subjects were to report any substance going into their body and not instructed concerning exact intake, the results of caloric intake were wide and varying. We believe we successfully mitigated the caloric intake as it pertains to influencing HRV by not allowing any food (fast), caffeine or nicotine 10 hours prior to the study. Consequences of food intake and metabolism fluctuations are not often recognized, however, consumption of food and water followed by gastric distension and bladder filling can directly impact HRV. Limiting food intake obviated this.

All of the subjects were requested to report any stress and/or stressful events in their lives in the 48 hours prior to the testing. While no significant stressful events were reported by any of the subject, minor daily stresses were reported. None of the subjects reported being at all stressed in the 10 hours preceding the test, nor during the test.

The tests were all administered between the hours of 08:00 and 10:00 am. This similar testing time should mitigate impacts due to differential restfulness and circadian
rhythm and its impact on HRV. None of the subjects reported being “tired” or lacking rest.

Each study participant was asked every 10 minutes throughout the study if they were “feeling ok” and if they “wished to proceed”. In all cases the participants signaled they wished to proceed with the study and that they were ok. Following the study, none of the participants had adverse responses or regrets about the study as polled in their exit interview.

Of all data collected, the most profound changes were observed during hypercapnic test periods in EtCO\textsubscript{2} and HRV SD2:SD1 ratio. Both EtCO\textsubscript{2} and HRV SD2:SD1 ratios changed by greater than 20%, in comparison to all other data groups. EtCO\textsubscript{2} monitoring is both expensive and requires a closed sampling of the expired gas. However, HRV calculation is simpler to perform utilizing more readily available portable ECG machines with the addition of a real-time software method of detection and analysis such as that developed here. One method of calculating the change is to develop an ellipse fitting algorithm which would remove obvious outliers in the data. Once that ellipse is projected around the data, a program could calculate the length of the short and long axis and compare it to the normal condition, as demonstrated in Figures 3.1-3.3 above. The graphs represented in Figures 3.1, 3.2 and 3.3 are representations of the computer program in Appendix A which was developed during the study to interpret the HRV return map plot. Such a software addition to standard ECG machines may offer the benefit of monitoring for hypercapnia-related HRV changes without the need for the additional cost and maintenance of EtCO\textsubscript{2} monitoring, and as such could be added to already existing ECG monitors in hospital or urgent care.
emergency departments, intensive care and pulmonary units, and to diver rebreather equipment.

The initial hypothesis of this research and development dissertation project was that the change in eccentricity of the ellipse fitted around the data would not increase the SD1 but decrease the SD2 bringing it closer to a one-to-one ratio. This assumption was predicated upon the initial HRV change data obtained from chronically ill patients. Surprisingly, the results from the present acute study indicate that the SD1 increases by as much as two times. Although the significant change in the responses due to the CO2 is noteworthy (as expected), the SD1 increase was not part of the initial assumption. However, the standard deviation ratio of SD2:SD1 did in fact change as predicted. A possible reason for this novel finding could be the body’s response to the stress imposed on it by the CO2. CO2 in and of itself is a vasodilator for the smooth muscles. As CO2 vasodilates, cardiac stroke volume increases which may have manifested the different presentation of the variance changes in HRV for the acute experiment carried out in the present dissertation.

We suspect that even given the increase in the SD1 by a significant amount that we observed in our clinical research experiment, as demonstrated on the right sides of figures 3.1-3.3 above, that if the exposure to CO2 continued for longer than the short term acute durations of time used here, that the HRV would remain in a similar “stressed ratio”, but would normalize and decrease in overall size to that commensurate with other distressed predictions on the right side of figure 1.3. Prolonged exposure to lower level amounts CO2 is a significant scientific and exploration obstacle on long-duration saturation diving missions as well as interplanetary travel and on the
International Space Station. The novel detection methodology developed in the present
dissertation bears significant credibility for carbon dioxide detection in chronic states as
well, although the changes due to elevated CO$_2$ appear to be different for acute vs.
chronic physiological states.

Given that HRV is predictive of a stressed ANS, there is a possibility that our new
systems and novel detection methodology could be predictive of other serious health-
related maladies. For instance, the ANS is stressed immediately preceding an epileptic
seizure. Since the mechanism of action in epilepsy is similar, not only could this be used
to sense an impending seizure before it starts, i.e., an early warning for an epileptic
seizure, but it could also be used to warn a diver of CNS O$_2$ toxicity seizures associated
with breathing hyperoxic gas mixtures. Since many undersea deaths have been blamed
on CNS O$_2$ toxicity this science could lend itself well to saving lives. Contrary to CO$_2$, O$_2$
is a vasoconstrictor. Because there is a possible use for this device to detect CNS O$_2$
toxicity and seizures, many tests would need to be conducted and the outcomes
assessed to better understand the body’s response to physiological stress and changes
in HRV.

4.1 Known Concerns and Issues to Overcome

We acknowledge that HRV monitoring can be affected by several confounding
factors. These factors include but are not limited to: sex, nicotine intake, caffeine intake,
several over-the-counter drugs, some prescription drugs, hormone levels, the presence
of oxidative stress, age, body mass index, depression, psychological stress, physical
activity levels and race [16,17]. HRV is also broadly impacted by cardiovascular,
vasoactive and psychotropic medications, circadian rhythms and wakefulness. Less
frequently recognized are the consequences of food intake and metabolism fluctuations. For example, it has been determined that the acute consumption of food and water, gastric distension and bladder filling can affect HRV [18]. For these reasons it is important for all users to become their own control in the use of this device. Gathering a consistent baseline calibration where-in a proposed device could “learn” the users predicted “normal” operating ellipse during a given exercise or activity, and then show a delta given the introduction of a specific pathology or stressor, are quite important experimental design features for clinical and commercial systems to be developed now.

While the results of the present experiment seem to be quite promising, it is evident that the data represent an initial positive outcome and bears further testing and development. Due to the stationary nature of each subject and the controlled environment, additional studies would be required before HRV monitoring can be recommended with a high degree of confidence for hypercapnia early detection monitoring in real-life environments. Additionally, HRV monitoring would have to be studied in symptomatic patients in comparison to standard EtCO$_2$ monitoring to see if HRV can accurately predict symptom onset as efficiently as standard EtCO$_2$ monitoring. That being said, if further experimentation confirms the provocative results reported here, there is significant potential for use in hospitals, outpatient surgery centers, first responder units, and pulmonary centers to monitor for symptoms associated with elevated levels of CO$_2$ in real time without the need for costly and timely arterial blood gas panels and EtCO$_2$ monitoring devices. Additionally, divers using rebreathers could benefit with real-time hypercapnia pre-detection. A waterproof version of the proposed device could be fitted to a diver’s suit and be effective at biometric monitoring both by
the diver and by a surface crew in training scenarios, thereby significantly increasing the overall diver’s safety and training.

4.2 Conclusion

Our initial clinical research cohort study demonstrated that HRV monitoring is reproducible in our study group, did show a statistically significant change in response to elevated inspired CO₂, and correlates with EtCO₂ monitoring. Our novel HRV methodology and algorithm can be easily added via software upgrades to any current ECG monitoring machines, or manufactured as a stand-alone ECG/HRV monitoring device. This type of monitoring can inexpensively reduce the need for additional, more expensive EtCO₂ monitoring devices and/or arterial blood gas analysis. We further propose that HRV analysis can provide an early warning system to detect hypercapnia related symptomatology prior to escalation of symptoms to a detrimental level and perhaps irreversible states in a number of health-related physiological stress scenarios and disease conditions.

4.3 Conclusions and Follow-on-Work

Follow-up research for this project would include development and testing of a more robust system which would be waterproof and incorporated into a smaller device that would integrate seamlessly with diving equipment or other medical monitoring and diagnosis systems. The next-generation system would have to accurately deal with additional parameters and factors such as sex, nicotine intake, caffeine intake, over-the-counter drugs, prescription drugs, hormone levels, the presence of oxidative stress, age, body mass index, depression, physiological stress, physical activity and possibly race. Additionally, further work and more testing needs to be done with a larger subject
group to incorporate and deal with the measurement noise that electromyography has on an ECG. A new more advanced system would need to have a learning algorithm which could learn the differences between each individual’s change in physical activity, which can reduce the HRV, as well as take into account stress on the ANS that is reducing the HRV. Furthermore, a concerted effort should be made to determine if the curved peak of a PPG could accurately determine HRV. This would allow incorporation into a smaller ring device or a device which shines an infrared light on the part of the ear between the concha and the antitragus in the area of the lower part of the ear. This is common for Special Operations operators to wear earbuds, and much of the general population wears earbuds regularly, making it an easy place to sense for HRV. It is apparent that the blood moves well in and out of these ear-related areas so those locations may be a good candidate site for tracking heart rate and changes in HRV.
REFERENCES


APPENDIX A: DITURI COMPUTER PROGRAM

GUI imports
import matplotlib
matplotlib.use("TkAgg")
from matplotlib.backends.backend_tkagg import FigureCanvasTkAgg,
NavigationToolbar2TkAgg
from matplotlib.figure import Figure
import matplotlib.animation as animation
from matplotlib import style
import Tkinter as tk
import numpy as np
from sklearn.neighbors import LocalOutlierFactor

# Imports for serial port
import serial
import Queue
import threading
import atexit
import copy
import time

# SERIAL PORT USED
COM_PORT = 'COM6'

# Set some styles
LARGE_FONT = ('Verdana', 12)
style.use('ggplot')
fig = Figure(figsize=(5,5), dpi=100)
axes = fig.add_subplot(111)
hl = []
program_is_running = True
no_plot_yet = True
first_r_time = True
N_VALUES_TO_SAVE = int(((10*60000)/200))
N_VALUES_TO_PLOT = 150
N_MIN_VALUES = 10
N_MIN_OUTLIERT_VALUES = 10 # Minimum values to start calculation of outliers
rr_times = np.zeros((N_VALUES_TO_SAVE))
r_timek = long()
r_timek_1 = long()
rr_k = 0.0
rr_k_1 = 0.0
ellipse = np.zeros((N_VALUES_TO_SAVE,2))
start_idx = 0
end_idx = 1

# Calculate the ratio of the axis of the ellipses for the time_min
def time_average_rr_ratio(time_min, rr_times, start_idx, end_idx):

time_ms = time_min*60000.0 # Minutes to ms conversion
# Construct linear array
if start_idx > end_idx:
    rr_data = np.concatenate((rr_times[start_idx:], rr_times[0:end_idx+1]), axis = 0)
else:
    rr_data = rr_times[start_idx:end_idx+1]
time_data = rr_data - rr_data
start_time_idx = np.argwhere( time_data > time_ms)
if time_ms == 0.0:
    # Calculate the full RR ratio
    rr_periods = np.diff(rr_data)
    n = len(rr_periods)
    #print('window: {}; nValues: {}'.format(time_min, n))
    #print(start_time_idx)
    if n > N_MIN_VALUES:
        # fit the outlier model
        clf = LocalOutlierFactor(n_neighbors = N_MIN_VALUES)
        y_pred = clf.fit_predict(rr_periods.reshape(-1,1))
inliers_idx = np.argwhere(y_pred > 0)
outliers_idx = np.argwhere(y_pred < 0)
rr_periods = rr_periods[inliers_idx]

# Parameters of the ellipse
n = len(rr_periods)
xs = rr_periods[0:n-2]
ys = rr_periods[1:n-1]

n = len(xs)
xc = np.mean(xs)
yc = np.mean(ys)

# Standard deviations from l1 and l2
"""See: Geometry of the Poincare plot of RR intervals and its asymmetry in healthy adults"""
SD1C = np.sqrt((1.0/(2.0*n))*np.sum(np.square((xs - ys) - (xc - yc))))

SD2C = np.sqrt((1.0/(2.0*n))*np.sum(np.square((xs + ys) - (xc + yc))))

# Standard deviation of the short axis of the ellipse to
# the standard deviation of the long axis of the ellipse
ratio = SD2C/SD1C
return ratio

else:
    return -1
else:
    # Check if enough time has passed to compute the require factor
    if np.shape(start_time_idx)[0] > 0:
        rr_periods = np.diff(rr_data[start_time_idx[-1,0]:-1])
        n = len(rr_periods)
        #print('window: {}; nValues: {}'.format(time_min, n))
        #print('start_time_idx: {}'.format(start_time_idx[0,0]))
        #print(start_time_idx[-1,0])
        if n > N_MIN_VALUES:
            # fit the outlier model
            clf = LocalOutlierFactor(n_neighbors = N_MIN_VALUES)
            y_pred = clf.fit_predict(rr_periods.reshape(-1,1))
            inliers_idx = np.argwhere(y_pred > 0)
            outliers_idx = np.argwhere(y_pred < 0)
            rr_periods = rr_periods[inliers_idx]

        # Parameters of the ellipse
        n = len(rr_periods)
        xs = rr_periods[0:n-2]
        ys = rr_periods[1:n-1]

        n = len(xs)
        xc = np.mean(xs)
        yc = np.mean(ys)

        # Standard deviations from l1 and l2
        # See: Geometry of the Poincare plot of RR intervals and its asymmetry in healthy adults
        SD1C = np.sqrt((1.0/(2.0*n))*np.sum(np.square((xs - ys) - (xc - yc))))
        SD2C = np.sqrt((1.0/(2.0*n))*np.sum(np.square((xs + ys) - (xc + yc))))
# Standard deviation of the short axis of the ellipse to
# the standard deviation of the long axis of the ellipse
ratio = SD2C/SD1C
return ratio
else:
    return -1
else:
    return -1

def update_plot(i):
    global hl, axes
    global start_idx, end_idx, ellipse
    global no_plot_yet

    # Calculate index extents
    n_first_values = end_idx + 1
    if start_idx > end_idx:
        if n_first_values > N_VALUES_TO_PLOT:
            plot_data = ellipse[end_idx - N_VALUES_TO_PLOT:end_idx + 1,:]
        else:
            plot_data = np.concatenate((ellipse[start_idx:,:],
                                         ellipse[0:n_first_values,:]), axis = 0)
    else:
        if n_first_values > N_VALUES_TO_PLOT:
            plot_data = ellipse[end_idx - N_VALUES_TO_PLOT:end_idx + 1,:]
        else:
            plot_data = ellipse[0:end_idx + 1,:]

    # fit the outlier model for plotting
    #print('plot_data shape (before): {}'.format(np.shape(plot_data)))
    if np.shape(plot_data)[0] > N_MIN_VALUES:
        clf = LocalOutlierFactor(n_neighbors = N_MIN_VALUES)
        y_pred = clf.fit_predict(plot_data)
        inliers_idx = np.argwhere(y_pred > 0)
        outliers_idx = np.argwhere(y_pred < 0)
        plot_data = plot_data[inliers_idx[:,0],:]
        #print('inliers_idx shape: {}'.format(np.shape(inliers_idx)))
        #print('plot_data shape: {}'.format(np.shape(plot_data)))

    # Check if this is the first time to plot
    if no_plot_yet:
        #hl, = axes.plot(plot_data[:,0], plot_data[:,1], 'ro')
        hl, = axes.plot(plot_data[:,0], plot_data[:,1], color = 'red', linestyle = 'None', alpha = 0.5,
marker = 'o', markeredgecolor = 'black', markersize = 5)
#hl, = axes.scatter(plot_data[:,0], plot_data[:,1], color = 'red',edgecolor='k', size = 20)
axes.set_xlabel(r'$R_{k}$ (ms)')
axes.set_ylabel(r'$R_{k-1}$ (ms)')
no_plot_yet = False
else:
    hl.set_xdata(plot_data[:,0])
    hl.set_ydata(plot_data[:,1])

# Set axis limits
axes.set_xlim([650, 1300])
axes.set_ylim([650, 1300])

def get_custom_time_window(event):
    global rrCustomTimeWindow
    print('Value entered: {}'.format(e1.get()))
    try:
        rrCustomTimeWindow = float(e1.get())
        label_rrCustomTitle.configure(text = "RR Custom: " + str(rrCustomTimeWindow) + " min")
    except ValueError:
        label_rrCustomTitle.configure(text = "RR Custom: NOT VALID!, using 1 min")
        rrCustomTimeWindow = 1.0

# Create window layout
# Create root window
root = tk.Tk()
root.title('RR Analysis')

# Create two frames
frame_left = tk.Frame(root)     # Contains the RR plot
frame_right = tk.Frame(root)    # Contains the indicators

frame_left.pack(side=tk.LEFT, fill=tk.BOTH, expand=True)
frame_right.pack(side=tk.RIGHT, fill=tk.BOTH, expand=True)

# Place plot title on left frame
msg_title = tk.Message(frame_left, text = 'RR Plot')
msg_title.config(bg = 'white', font = ('times',24,'italic'), width = 400)
msg_title.pack()

# Create canvas for plot
canvas = FigureCanvasTkAgg(fig, master = frame_left)
canvas.show()
#canvas.get_tk_widget().grid(row = 1, column = 0)
canvas.get_tk_widget().pack(side=tk.BOTTOM, fill=tk.BOTH, expand=True)

toolbar = NavigationToolbar2TkAgg(canvas, frame_left)
toolbar.update()
canvas._tkcanvas.pack(side=tk.TOP, fill=tk.BOTH, expand=True)

# Place indicators on right frame
label_rr1minTitle = tk.Label(frame_right, text = 'RR: 1 min', relief = 'ridge', width=15)
label_rr1minTitle.pack(side=tk.TOP, fill=tk.BOTH, expand=True)
rr1minValueDouble = np.nan
rr1minValueString = tk.StringVar()
rr1minValueString.set('-')
msg_rr1minValue = tk.Message(frame_right, textvariable = rr1minValueString)
msg_rr1minValue.pack(side=tk.TOP, fill=tk.BOTH, expand=True)

label_rr5minTitle = tk.Label(frame_right, text = 'RR: 5 min', relief = 'ridge', width=15)
label_rr5minTitle.pack(side=tk.TOP, fill=tk.BOTH, expand=True)
rr5minValueDouble = np.nan
rr5minValueString = tk.StringVar()
rr5minValueString.set('-')
msg_rr5minValue = tk.Message(frame_right, textvariable = rr5minValueString)
msg_rr5minValue.pack(side=tk.TOP, fill=tk.BOTH, expand=True)

label_rr10minTitle = tk.Label(frame_right, text = 'RR: 10 min', relief = 'ridge', width=15)
label_rr10minTitle.pack(side=tk.TOP, fill=tk.BOTH, expand=True)
rr10minValueDouble = np.nan
rr10minValueString = tk.StringVar()
rr10minValueString.set('-')
msg_rr10minValue = tk.Message(frame_right, textvariable = rr10minValueString)
msg_rr10minValue.pack(side=tk.TOP, fill=tk.BOTH, expand=True)

label_rrFullTitle = tk.Label(frame_right, text = 'RR: Full', relief = 'ridge', width=15)
label_rrFullTitle.pack(side=tk.TOP, fill=tk.BOTH, expand=True)
rrFullValueDouble = np.nan
rrFullValueString = tk.StringVar()
rrFullValueString.set('-')
msg_rrFullValue = tk.Message(frame_right, textvariable = rrFullValueString)
msg_rrFullValue.pack(side=tk.TOP, fill=tk.BOTH, expand=True)

label_rrCustomTitle = tk.Label(frame_right, text = 'RR: Custom 1 min', relief = 'ridge', width=15)
label_rrCustomTitle.pack(side=tk.TOP, fill=tk.BOTH, expand=True)
# Create an entry for the custom RR ratio time window
e1 = tk.Entry(frame_right)
e1.bind("<Return>", get_custom_time_window)
e1.pack(side=tk.TOP, fill=tk.BOTH, expand=True)
rrCustomTimeWindow = 1.0
rrCustomValueDouble = np.nan
rrCustomValueString = tk.StringVar()
rrCustomValueString.set('-')
msg_rrCustomValue = tk.Message(frame_right, textvariable = rrCustomValueString)
msg_rrCustomValue.pack(side=tk.TOP, fill=tk.BOTH, expand=True)

# Create threads for serial port and for updating the plot
queue = Queue.Queue(100)

# This read each line arrived from the serial port
def serial_read(ser):
    global program_is_running, queue
    
    # Verify the port is available
    ser.baudrate = 9600
    ser.port = COM_PORT
    ser.timeout = 1
    ser.open()  # Open serial port. CATCH EXCEPTION
    while program_is_running:  # While serial port is opened
        line = ser.readline()
        if line != '':
            queue.put(line)
    ser.close()
    print('Ending thread: serial_read\n')

# This extracts the R time from each line arrived from the serial port
def calculate_rr():
    global rr1minValueString, queue, k
    global r_timek, r_timek_1, rr_k, rr_k_1, rr_intervals
    global k, xList, yList, hl, axes, start_idx, end_idx
    global rr_times, first_r_time
    
    while program_is_running:  # While program is running
        line = queue.get(block = True)#, timeout = None)
        if (line != '') and (line != 'EndThread'):
            # Update values
            s, r_time = line.split(',')
            r_timek = float(r_time)
            if first_r_time:  # Check if this is the first time
rr_times[0] = r_timek
rr_k = 0.0
rr_k_1 = 0.0  # r_time
first_r_time = False
else:
    rr_k = float((r_timek - r_timek_1))

#rr_intervals[end_idx] = rr_k
rr_times[end_idx] = r_timek
ellipse[end_idx, :] = np.array([rr_k, rr_k_1])

rr1minValueDouble = time_average_rr_ratio(1.0, rr_times, start_idx, end_idx)
if rr1minValueDouble != -1:
    string = '{:.2f}'.format(rr1minValueDouble)
    rr1minValueString.set(string)
else:
    rr1minValueString.set('-')

rr5minValueDouble = time_average_rr_ratio(5.0, rr_times, start_idx, end_idx)
if rr5minValueDouble != -1:
    string = '{:.2f}'.format(rr5minValueDouble)
    rr5minValueString.set(string)
else:
    rr5minValueString.set('-')

rr10minValueDouble = time_average_rr_ratio(10.0, rr_times, start_idx, end_idx)
if rr10minValueDouble != -1:
    string = '{:.2f}'.format(rr10minValueDouble)
    rr10minValueString.set(string)
else:
    rr10minValueString.set('-')

rrFullValueDouble = time_average_rr_ratio(0.0, rr_times, start_idx, end_idx)
if rrFullValueDouble != -1:
    string = '{:.2f}'.format(rrFullValueDouble)
    rrFullValueString.set(string)
else:
    rrFullValueString.set('-')

rrCustomValueDouble = time_average_rr_ratio(rrCustomTimeWindow, rr_times, start_idx, end_idx)
if rrCustomValueDouble != -1:
    string = '{:.2f}'.format(rrCustomValueDouble)
    rrCustomValueString.set(string)
else:
    rrCustomValueString.set('-')
# Update the indices of the circular buffer
if end_idx >= start_idx:
    end_idx = end_idx + 1
else:
    end_idx = end_idx + 1
    start_idx = start_idx + 1

if end_idx == N_VALUES_TO_SAVE-1:
    end_idx = 0
    start_idx = 1

# Update variables for the next iteration
r_timek_1 = r_timek
rr_k_1 = rr_k

print('time (ms) = {}'.format(r_timek))

def on_closing(serial_port_handle):
    global program_is_running, queue

    program_is_running = False
    serial_port_handle.cancel_read()
    queue.put('EndThread')  # Put empty line to unblock thread
    time.sleep(1)
    root.destroy()

# Create serial port object
serial0 = serial.Serial()

# Start threads
thread1 = threading.Thread(target=serial_read, args = (serial0,)).start()# args = (serial0,)).start()
thread2 = threading.Thread(target=calculate_rr).start()

# Setup the updating screen
ani = animation.FuncAnimation(fig, update_plot, interval = 300)
root.protocol("WM_DELETE_WINDOW", lambda arg0 = serial0: on_closing(arg0))  # Closing actions
root.mainloop()

""cd "C:\Users\dituri\Documents\PythonCode\ecg_project"
python rr_analysis_f_v1.py""
APPENDIX B: GLOSSARY OF TERMS

ABG – Arterial Blood Gas
ADC – Analog-to-digital converter
ANS – Autonomic Nervous System
APPS – Applications
BMI – Body Mass Index
CCR – Closed Circuit Rebreather
CNS – Central Nervous System
CO₂ – Carbon Dioxide
COPD – Chronic Obstructive Pulmonary Disease
CPR – Cardiopulmonary Resuscitation
DIN – Deutsches Institut für Normung
Dead air space – volume of air which is inhaled that does not take part in the gas exchange.
ECG – Electro Cardiogram
FPU – Floating-point Unit
HF – High Frequency
HRV – Heart Rate Variability
IRB – Institutional Review Board
LED – Light Emitting Diode
LF – Low Frequency
LF/HF – Low Frequency Divided by High Frequency
MCU – Microcontroller unit
O₂ – Oxygen
OTC – Over the Counter
Poincaré plot – Return map plot popularized by Poincare.
PPG – Photoplethysmography
PSI – Pounds per Square Inch
PaCO₂ – Carbon dioxide arterial pressure
Rebreather – Recirculation device
RR – Distance from the R of the QRS complex to next R
SD – Standard Deviation
SD1 – Proposed short side of the HRV Poincaré plot ellipse
SD2 – Proposed longer side of the HRV Poincaré plot ellipse
SCUBA – Self Contained Underwater breathing Apparatus
SCR – Semi-Closed Rebreather
SPI – Serial Peripheral Interface
TFT LCD – Thin-film-transistor liquid-crystal display
APPENDIX C: INFORMED CONSENT FORM

Informed Consent to Participate in Research and Authorization to Collect, Use, and Share your Health Information

You are being asked to take part in a research study. Research studies include only people who choose to take part. This document is called an informed consent form. Please read this information carefully and take your time making your decision. Ask the researcher or study staff to discuss this consent form with you. Please ask him/her to explain any words or information you do not clearly understand. We encourage you to talk with your family and friends before you decide to take part in this research study. The nature of the study, risks, inconveniences, discomforts, and other important information about the study are listed below.

We are asking you to take part in a research study called: Novel Heart Rate Variability and CO2, Real-time Detection and Measurement System

The person who is in charge of this research study is Dr. Robert Frisina. This person is called the Principal Investigator. However, other research staff may be involved and can act on behalf of the person in charge. Joseph Dituni is the co-principal investigator and study coordinator.

The research will be conducted at IBUM Facility at 701 N. Westshore Blvd Tampa, FL 33609.

This research is being sponsored by the investigators.

Purpose of the study

Develop a method for testing heart rate variability (HRV) to determine if it can predict hypercapnia. The heart generally beats 60 beats per minute. Really it beats between 700 milliseconds (ms) and 1400 ms which is a little more and a little less than 1 beat per second. The HRV is the variability between 700 and 1400 ms. HRV may be able to be used as a method to measure stress on the body. A high level of HRV is linked to good health and a low level HRV is linked to stress/fatigue and poor health.

Why are you being asked to take part?

We are asking you to take part in this research study because we want to advance the science of CO2 detection. This study includes the use of a 12 lead Electrocardiogram (ECG) to monitor the variations in heart rate as a predictor of hypercapnia or too much CO2.

Study Procedures: What will happen during this study?

- At least 48 hour prior to the study each participant will receive a physical examination by a physician.
- All subjects shall keep a lifestyles diary during the study with food intake, nicotine intake, caffeine intake, drugs, sports or dietary supplements, alcohol and any medications taken for 48 hours prior to the study period. Additional entries shall be made in the diary concerning stresses experienced within 10 hours of the study.
- All subjects will be required to fast (go without food) for 10 hours prior to participation. Water is acceptable at any time during the fast.
- Additionally, all subjects shall not perform exercise for 10 hours prior to participation.
- Nicotine and caffeine are not allowed during the 10-hour fast prior to the study.
• Each subject shall go through an identical intake process for each subject consisting of notification of subject's rights, discussion and signature of consent forms along with a relaxation period.
• On the day of the study, subjects will enter into the testing room and lay upon the subject table in a dimly lit silent room.
• The technician (Joseph Dittoni) shall connect the ECG leads to the subjects and connect them to the breathing apparatus set to deliver normal air.
• The physician will be in the room as a second monitor for any medical issues.

  o An adjustment period should be afforded to help those not comfortable with the breathing device. This period will have low ambient room lighting, similar room temperature and no loud noises or visual disturbances while connected to the ECG.
  o The room conditions shall remain the same during all testing throughout the study.
  o There will be three 10-minute exposures to high CO₂ breathing periods which are programmed to be air, 4%, 5% and then 6% carbon dioxide all of which are followed by a recovery period.
  o Exposure levels of CO₂ are to be 4%, 5%, and 6% are shown in Table 1 below.
  o Each cycle will start with a 20-minute warm up period followed by an increasing exposure to CO₂ for 10 minutes followed by a 30 minute breathing air (recovery period) or until the CO₂ is completely washed out followed by another 10 minute breathe predetermined mix to assess post-CO₂. This will continue for 4 cycles breathing all the gasses listed in Table 1 in a set up (increasing) order of CO₂.
  o The entire study shall be recorded with video and audio settings to monitor and record any symptoms of CO₂ toxicity. The Principal investigator and his staff shall have access to the video and audio and will review same to determine subtle high CO₂ symptoms. The video and audio shall be electronically deleted upon publication of the final dissertation. There will be no change in normal medical treatment following this participation.
  o An initial visit which will take approximately and hour is required for the physical exam and paperwork to include a neurological assessment as well as a spirometry test. At least 48 hours later, you will have one visit with the person in charge of the study or study staff. Most study visits will take about 4 hours. A physical will be administered following completion of the study on the same day and the subject will be released. No other visits are required.

```
<table>
<thead>
<tr>
<th>Mixture gas content</th>
</tr>
</thead>
<tbody>
<tr>
<td>21% O₂, 79% N₂</td>
</tr>
<tr>
<td>21% O₂, 4% CO₂, 71% N₂</td>
</tr>
<tr>
<td>21% O₂, 5% CO₂, 72% N₂</td>
</tr>
<tr>
<td>21% O₂, 6% CO₂, 73% N₂</td>
</tr>
</tbody>
</table>
```

Table 1

**Total Number of Participants**
About 15 individuals will take part in this study at the International Board of Undersea Medicine.

**Alternatives / Voluntary Participation**
You do not have to participate in this research study. You should only take part in this study if
Compensation for Research Related Injuries

If you are experiencing an emergency, call 911. If you believe you have been harmed as a result of participating in this study, you should call Joseph Ditunno at (202) 642-3483 as soon as possible. The University of South Florida has not set aside money to pay for illness or injury that may result from your participation in research. The cost of such care will be billed to your insurance company or to you in the event you do not have medical insurance. Before you agree to take part in this study, you may want to find out whether your insurance will cover injuries that result from taking part in research. You may be responsible for any deductible, co-insurance, or co-payments that result from such care. If you are injured, the University of South Florida has also not set aside money for lost wages, discomfort or disability you may experience as a result of a research related injury. You do not give up your legal rights by signing this form. In addition to contacting the study investigator, you should also contact the USF Institutional Review Board (IRB) at 813-974-5638 or by email at RSCH-IRB@usf.edu, if you believe you have been injured as a result of taking part in this study.

Compensation

You will be compensated $25 if you complete all the scheduled study visits. If you withdraw for any reason from the study before completion you will be compensated $25.

Previous similar CO2 studies have shown no long-term impact medically. Medical care for research related injuries is not covered or offered.

The findings from this research may result in the future development of products that are of commercial value. There are no plans to provide you with financial compensation or for you to share in any profits if this should occur.

Costs

It will not cost you anything to take part in the study.

Privacy and Confidentiality

We will keep your study records private and confidential. Certain people may need to see your study records. Anyone who looks at your records must keep them completely confidential. These individuals include:

- The research team, including the Principal Investigator, physicians, study coordinator, research nurses, and all other research staff.
- Certain government and university people who need to know more about the study. For example, individuals who provide oversight on this study may need to look at your records. This is done to make sure that we are doing the study in the right way. They also need to make sure that we are protecting your rights and your safety.
- Any agency of the federal, state, or local government that regulates this research. This includes the Department of Health and Human Services (DHHS) and the Office for Human Research Protection (OHRP).
- The USF Institutional Review Board (IRB) and its related staff who has oversight responsibilities for this study, and staff in USF Research Integrity and Compliance.
- The International Board of Undersea Medicine

We may publish what we learn from this study. If we do, we will not include your name. We will not
publish anything that would let people know who you are.

**What if new information becomes available about the study?**
During the course of this study, we may find more information that could be important to you. This includes information that, once learned, might cause you to change your mind about being in this study. We will notify you as soon as possible if such information becomes available.

**You can get the answers to your questions, concerns, or complaints.**
If you have any questions, concerns or complaints about this study, call Joseph Druci at 202-642-3483. If you have questions about your rights, complaints, or issues as a person taking part in this study, call the USF IRB at (813) 974-5638 or contact by email at RSCH-IRB@usf.edu.

**Authorization to Use and Disclose Protected Health Information (HIPAA Language)**
The federal privacy regulations of the Health Insurance Portability & Accountability Act (HIPAA) protect your identifiable health information. By signing this form, you are permitting the University of South Florida to use your health information for research purposes. You are also allowing us to share your health information with individuals or organizations other than USF who are also involved in the research and listed below. In addition, the following groups of people may also be able to see your health information and may use that information to conduct this research:

- The medical staff that takes care of you and those who are part of this research study;
- The research site for this study;
- Data Safety Monitoring Boards or others who monitor the data and safety of the study;
- The USF Institutional Review Board (IRB) their related staff who have oversight responsibilities for this study, including staff in USF Research Integrity and Compliance and the USF Health Office of Clinical Research.

Anyone listed above may use consultants in this research study, and may share your information with them. If you have questions about who they are, you should ask the study team. Individuals who receive your health information for this research study may not be required by the HIPAA Privacy Rule to protect it and may share your information with others without your permission. They can only do so if permitted by law. If your information is shared, it may no longer be protected by the HIPAA Privacy Rule.

By signing this form, you are giving your permission to use and/or share your health information as described in this document. As part of this research, USF may collect, use, and share your research record.

You can refuse to sign this form. If you do not sign this form you will not be able to take part in this research study. However, your care outside of this study and benefits will not change. Your authorization to use your health information will not expire unless you revoke (withdraw) it in writing. You can revoke this form at any time by sending a letter clearly stating that you wish to withdraw your authorization to use your health information in the research. If you revoke your permission:

- You will no longer be a participant in this research study;
- We will stop collecting new information about you;
- We will use the information collected prior to the revocation of your authorization. This information may already have been used or shared with others, or we may need it to complete and protect the validity of the research, and
Staff may need to follow-up with you if there is a medical reason to do so.

To revoke this form, please write to:
Joseph Dituri
701 N. Westshore Blvd Tampa, FL 33602
For IRB Study # [Pro00030609]

While we are conducting the research study, we cannot let you see or copy the research information we have about you. After the research is completed, you have a right to see the information about you, as allowed by USF policies.

Consent to Take Part in Research

Consent to Take Part in Research
And Authorization for the Collection, Use and Disclosure of Health Information
I freely give my consent to take part in this study and authorize the use of my health information as outlined above. I understand that by signing this form I am agreeing to take part in research. I have received a signed copy of this form to take with me.

Signature of Person Taking Part in Study

Date

Printed Name of Person Taking Part in Study

Statement of Person Obtaining Informed Consent and Research Authorization
I have carefully explained to the person taking part in the study what he or she can expect from their participation. I confirm that this research subject speaks the language that was used to explain this research and is receiving an informed consent form in their primary language. This research subject has provided legally effective informed consent.

Signature of Person Obtaining Informed Consent

Date

Printed Name of Person Obtaining Informed Consent
APPENDIX D: DAILY FOOD LOG

Daily food / drink / supplement diary

If you put it in your body in any way, please note it on this sheet for 48 hours prior to the study.

Your study date is _________________.
Start your log on _________________.

<table>
<thead>
<tr>
<th>Item</th>
<th>Time / Date</th>
<th>Approximate Quantity</th>
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APPENDIX E: DATA COLLECTION SHEETS

The following information will be digitally recorded by the devices supplied:
- ETCO₂ Digital
- Video / Audio

Entire cardiac rhythm digitally (This will be converted into HRV, QRS and any other data required)

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<th>+2 Min</th>
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<th>+8 min</th>
<th>+10 min</th>
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**Physical examination General format**

Age _____ Height _____ Weight _____

**GENERAL APPEARANCE:** The patient is alert and oriented and in no acute distress.

**VITALS SIGNS:** Temperature _____, pulse ___, respirations ____, and blood pressure is ____.

**HEENT:** Head is normocephalic with normal hair distribution. No evidence of trauma. Ears: No acute purulent discharge. Eyes: Conjunctivae pink with no scleral jaundice. Nose: Normal mucosa and septum.

**NECK:** Supple with no cervical or supraclavicular lymphadenopathy. Trachea is midline. Thyroid: Not palpable.

**LUNGS:** Normal symmetrical expansion of both hemithoraces. Coarse breath sounds with some rhonchi.

**HEART:** S1 and S2 normal.

**ABDOMEN:** Soft.

**EXTREMITIES:** No swelling or effusion in any of the joints of the hands or feet. No peripheral edema.

**SKIN:** Normal color, turgor and temperature. No ulcerations or rashes noted.

**LYMPHATICS:** No cervical or supraclavicular lymphadenopathy.

**NEUROLOGICAL:** Alert and oriented. GCS is ____.
Neurological checklist

Patient Name ____________________________
Examiner Name __________________________ Date/Time of Exam _________________

Pain Areas - Shade with XX s

ASIA STANDARD NEUROLOGICAL CLASSIFICATION OF SPINAL CORD INJURY

MOTOR

KEY MUSCLES

(Lossing or reverse of a line)

C5
C6
C7
C8
T1
Elbow flexors
Wrist extensors
Elbow extensors
Finger flexors (anterior phalanx of thumb)
Finger abduction (little finger)

UPPER LIMB TOTAL

MAXIMUM

(25) (25) (50)

LOWER LIMB TOTAL

MAXIMUM

(25) (25) (50)

Sensory Points

Key

Voluntary anti-contraction (Yes/No)

Any anal sensation (Yes/No)

PIN PRICK SCORE (max 12)

LIGHT TOUCH SCORE (max 12)

NEUROLOGICAL LEVEL

SENSORY MOTOR

COMPLETE OR INCOMPLETE?

ZONE OF PARTIAL PRESERVATION

ASIA IMPAIRMENT SCALE

58
# IANTD Field Neurological Checklist

## Metal Status:
- Time and Place: Circle, Y/N
- 3 Object Memory: Circle, Y/N
- States Situation: Circle, Y/N
- Performs Serial 7’s: Circle, Y/N
- Confusion: Circle, Y/N

## Cranial Nerves:
- Tongue Midline: Circle, Y/N
- Nystagmus Present: Circle, Y/N
- Eyes Move 4 Directions: Circle, Y/N
- Hearing Equal: Circle, Y/N
- Ringing Ears: Circle, Y/N
- Pupils Equal: Circle, Y/N
- Larger Pupil: Circle, R/L /=

## Coordination:
- Romberg Normal: Circle, Y/N
- Heel Toe Walk Passed: Circle, Y/N
- Finger Nose Passed: Circle, Y/N
- Duck Walk Capable: Circle, Y/N
- Heel Shin Slide Pass: Circle, Y/N

## Sensory
- Numbness/tingling (Torso): Circle, Y/N
- Numbness/tingling (Extremity): Circle, Y/N
- Pain (Torso): Circle, Y/N
- Pain (Joint): Circle, Y/N
- Radiating/ridiculer: Circle, Y/N
- Other: Circle, Y/N

## Strength
- Weakness
  - Bilateral: Circle, Y/N
  - One side: Circle, Y/N
- Fatigue: Circle, Y/N

## DTR
- Difference bilaterally: Circle, Y/N
- Non-responsive: Circle, Y/N

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## Additional Notes:

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APPENDIX F: IRB APPROVAL

8/21/2018

Joseph Dituri, MS
Chemical and Biomedical Engineering
Tampa, FL 33602

RE: Full Board Approval for Continuing Review
IRB#: CR1_Pro00030609
Title: Novel Heart Rate Variability and CO2, Real-time Detection and Measurement System

Study Approval Period: 8/7/2018 to 8/7/2019

Dear Mr. Dituri

On 8/6/2018, the Institutional Review Board (IRB) reviewed and APPROVED the above application and all documents contained within, including those outlined below.

Approved Item(s):
Protocol Document(s):
Clean copy version 1 dot 4

Consent/Assent Document(s)*:
BioMed Adult Consent form 8 10 17 Clean copy.pdf

*Please use only the official IRB stamped informed consent/assent document(s) found under the "Attachments" tab on the main study's workspace. Please note, these consent/assent document(s) are valid until they are amended and approved.

As the principal investigator of this study, it is your responsibility to conduct this study in accordance with USF HRPP policies and procedures and as approved by the USF IRB. Any changes to the approved research must be submitted to the IRB for review and approval via an amendment. Additionally, all unanticipated problems must be reported to the USF IRB within five (5) business days.

We appreciate your dedication to the ethical conduct of human subject research at the University of South Florida and your continued commitment to human research protections. If you have any questions regarding this matter, please call 813-974-5638.
ABOUT THE AUTHOR

CDR Joseph Dituri enlisted in the U.S. Navy in 1985. He served continuously on active service upon various ships and shore stations where he was involved in hyperbaric system certification, saturation diving and ship repair. In 1995 he made his way up through the ranks after earning his B.S. in Computer Science from the University of South Carolina and was commissioned into the Special Operations Officer pipeline. He then attended Naval Post Graduate School where he earned his Master's degree in Astronautical Engineering. His master's thesis topic was in Orbital Determination with an accent for life support systems. He is an invited speaker on space related topics.

Now that he is retired from almost 28 years of active service to the United States, he is a consultant for the International Board of Undersea Medicine. He also volunteers his time as the CEO of the Association for Marine Exploration. His research areas of interest include life support equipment, high carbon dioxide environments as well as hyperbaric and hypobaric medicine. Joseph has 3 daughters and enjoys writing books, skydiving and has had a lifelong goal of being a civilian astronaut.