Measuring Compartment Size and Gas Solubility in Marine Mammals

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LONG-TERM GOALS

The long term goal of this study is to develop methods to estimate marine mamal tissue compartment sizes, and tissue gas solubility. We aim to improve the data available for the relative size of different tissues in various marine mammal species, as well as our understanding of their different morphological and physiological adaptations. The study will also develop a method that enables the determination of the gas solubility in different tissue compartments.

OBJECTIVES

This study include two main objectives: to study the morphometrics of marine mammal compartments and the solubility coefficient of these compartments. Both objectives need the development of new methods to reach their respective goals.

The first objective is aimed at improving the data available for the relative size of different tissues in various marine mammal species, as well as our understanding of the different morphological and physiological adaptations that exist among marine mammals. Previous efforts have been focused on measuring the major O_2 stores, such as muscle mass and myoglobin (Mb) concentration, or total blood volume and hemoglobin content (Ponganis et al., 2011). There is also little or no information for certain tissue compartments such as skin, blubber, muscle, heart, lung, liver, kidneys, spleen or bone. The relative size of each compartment has not been properly calculated with a consistent methodology. Therefore there is a need to consistently measure the relative size of the different tissues: such as skin, muscle, blubber, heart, and lungs in as many species as possible.

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The second objective is aimed at developing a method that enables the determination of the gas solubility in different compartments. There are limited data on gas solubility in marine mammal tissues: species differences have been found and variations compared to land mammals are expected (Koopman and Westgate, 2012). We aim to modify the Koopman et al. (2012) method to enable the study of gas solubility from "solid" tissues (skin, blubber, muscle, brain, liver, kidney) and we are also going to study the solubility of all gases (N_2, O_2, CO_2 and H_2) that are routinely found in bubbles of stranded marine mammals (Bernaldo de Quirós, 2011; Bernaldo de Quirós et al., 2012). This modification will consist of an adaptation of the Scholander method for measuring tissue gas content (Scholander, 1942). Therefore we will also be able to analyze the gas composition of tissues of marine mammals. Within this project, we will determine the "original" gas composition of the tissues of a given animal and we will analyze the relationship between tissue gas content to amount and composition of gas bubbles found in different tissues by using the methods developed by Bernaldo de Quirós et al. (2012; 2011), in addition to gas solubility studies.

APPROACH

OBJECTIVE 1

Aim 1: Obtaining morphometric data of different species. For this aim fresh specimens of adult animals will be requested from different locations: North Carolina, Cape Cod Bay and from the Canary Islands. In addition, access to bycaught animals will be facilitated by NOAA. A mass dissection protocol to systematically separate the body into discrete anatomical components will be developed in collaboration with McLellan and Pabst, based on their previous experience (McLellan et al., 2002). Tissues will be weighed separately. Volume will be measured by water displacement. Density will be calculated by dividing the weight by the volume. Finally, we will report the mass of each body compartment as a percentage of the total body mass in accordance to Grand (1977).

Aim 2: Muscle myoglobin determination. Myoglobin content will be calculated for the different muscle groups, including heart, of each specimen following the method described by Polasek and Davis (2001). Dr. Pabst will introduce Dr. Bernaldo de Quiros in this technique.

OBJECTIVE 2

Aim 1: Design of an anaerobic tissue grinder. In 1942, Scholander designed a device for the determination of the gas content in tissues (Scholander, 1942). We will modify this device to allow quick removal of the tissue. This way, removal and grinding of the tissue will be done as anaerobically as possible. We will design the device in such way that blood, water and other liquids will be separated from the tissue. The ground tissue will be transferred to an anaerobic glass tube. We have already been in contact with WHOI engineers that have assured us that this modification is feasible.

Aim 2: Determine the tissue solubility coefficient of gases. We will follow Koopman et al. (2012) instructions; although some modifications will be needed in order to study other tissues than blubber and other gases than N_2. The development of this method will be done in collaboration with Dr. Sylva and Dr. Seewald from WHOI and Dr. González Díaz form the Canary Islands. The method will be applied at WHOI and in the canaries (at the ULPGC). We will validate our method by running samples for which solubility coefficients have been previously reported such as water and olive oil (Weathersby and Homer, 1980). Once we know the method is generating accurate results, we will determine the solubility coefficient of the gases of interest in the tissues for which morphometrics are measured. We are aware of the complexity of this aim and therefore plan more than one year to develop these techniques and complete this aim.

Aim 3: Analyzing gas content in tissues. Once tissues have been transferred anaerobically, we will analyze the gas content using the headspace method. The headspace is the vapor in equilibrium with its liquid phase. When a dissolved substance is sufficiently volatile, the determination of its concentration in the vapor phase can be used as a measure of the concentration in the liquid phase if the solubility coefficient is previously known, providing that equilibrium between the vapor and liquid phases has been reached. It will be very interesting to see what the actual gas tension of the different gasses in marine mammals is and how it relates to gas composition in the bubbles (Bernaldo de Quirós et al., 2012).

WORK COMPLETED

The project began June 17 2013. A subaward to TAMUCC has been encumbered and we are coordinating project plans with the four different institutions: WHOI, TAMUCC, UNCW, and the ULPGC. Logisitcs for the planned experiments and acquisition of materials to begin the work are underway. In addition, to plan the development of the different methods, we are gathering additional reference sources.

RESULTS

No results are yet available.

IMPACT/APPLICATIONS

Prior work has suggested that marine mammals are commonly supersaturated with gas, such that a direct ascent to the surface result in bubble formation in most tissues (Moore et al., 2009). Recent work by Bernaldo de Quiros et al (2012) has shown that gas composition analysis can discriminate between gas from decompression as opposed to decomposition. Fresh, drowned-at-depth ascended bycatch do indeed show evidence of postmortem decompression from a supersaturated state (Bernaldo de Quirós et al., 2013). How do marine mammals normally avoid DCS symptoms when at the surface? This proposal will help improve the parameters used for modeling gas management in marine mammals and improve understanding of how these animals manage gases while diving and breathing at the surface. A better understanding of their normal physiology is required to answer this question and will help determine how they normally avoid DCS.

RELATED PROJECTS

This project is related to N000141210388 'Markers of decompression stress of mass stranded/live caught and released vs. single stranded marine mammals' where we are using a biomarker to examine bubble stress on neutrophils and endothelial cells in diving marine mammals, in collaboration with Dr Stephen Thom at the University of Maryland.

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