Proposed By:

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Title: Measurement of Diaphragm Blood Flow Using Laser Doppler Flowmetry

This research project will be investigating the use of Laser Doppler Flowmetry (LDF) for continuous measurement of diaphragm perfusion. The instrument efficiency will be determined by the ability of the instrument to measure expected and manipulated changes in diaphragm perfusion. Supportive results will be used in Dr. Smith-Blair's research on emphysema and diaphragm function. This will contribute to a greater body of knowledge on diaphragm perfusion, which will be of great benefit to understanding treatment modalities in pulmonary disease and the body of knowledge concerning the effect of emphysema on diaphragm fatigue.

Measurement of Diaphragm Blood Flow Using Laser Doppler Flowmetry

The diaphragm is the major ventilatory muscle. Diaphragm fatigue has a major role in the pathogenesis of many respiratory diseases. An important aspect in the development of diaphragm fatigue is related to the ratio between energy requirements versus energy supplied. Resting energy stores are minimal and are able to sustain the diaphragm for only a few seconds. Therefore, the ability of the diaphragm to receive adequate diaphragmatic blood flow is essential in preventing diaphragm fatigue. Methods enabling measurement of diaphragm blood flow are of great interest.

The impaired blood flow to the diaphragm may be explained by the decreased diaphragmatic perfusion pressure from the added extrinsic load on the diaphragm and increased vascular resistance from higher vessel tortuosity in a shortened muscle. Changes in tissue blood flow can cause impaired perfusion which can contribute to the development of diaphragm dysfunction and respiratory failure. Previous methods to evaluate diaphragm blood flow included complex procedures using xenon clearance, radio-labeled or florescent microsphere techniques, or video-microscopy. Recently the use of laser Doppler flowmetry (LDF) has been developed that allows continuous recording of relative changes in local blood flow. The LDF has been used to assess microcirculation in various animal and human tissues but only two studies have examined the use of laser Doppler flowmetry in the diaphragm.

The purposes of this study are to evaluate use of LDF in detecting changes in basal microcirculation and pharmacologically induced hyperemia in the *in vivo* hamster model. The aims of the study are:

AIM 1: To evaluate the use of LDF in detecting changes in basal microcirculation during contraction and relaxation of the diaphragm muscle.

Hypothesis 1. Changes in diaphragm blood flow can be detected as changes in LDF.

AIM2: To investigate the use of LDF in detecting pharmacologically induced changes in blood flow in the diaphragm.

Hypothesis 1. Intravenous infusion of butoxamine, a B_2-adrenoreceptor antagonist will result in decreased LDF measurements in the diaphragm muscle.

Hypothesis 2. Intravenous infusion of salbutamol, a B_2-adrenoreceptor agonist, will increase LDF measurements in the diaphragm muscle.

Background/Significance: In emphysema, the diaphragm is affected by lung hyperinflation that causes it to be displaced and become shorter than normal which causes the capillary network become tortuous. Consequently, in addition to the inspiratory muscles in emphysema being characterized by a discrepancy between load and capacity the perfusion of the capillary beds may also suffer. The impaired diaphragm shortening and reduced blood flow can contribute to the development of diaphragm fatigue. Despite the importance of diaphragm blood flow, there is little information concerning the characteristics of *in vivo* diaphragmatic blood flow.

Diaphragm blood flow: The diaphragm is supplied by three main arteries: the internal mammary, the intercostals, and the inferior phrenic arteries. Additionally, there is a vast anastomotic network between these arteries so that blood supply remains relatively constant even when vascular occlusion of one of the vessels occurs (6).

Blood flow during diaphragm activity: Diaphragm performance depends on diaphragm blood flow. Blood flow to the diaphragm increases with increasing muscular activity and noted to be maintained relatively constant over a wide range of perfusion pressures (3). Poole and associates (1992) demonstrated that increasing blood flow to the diaphragm during hypotensive states can reverse diaphragm fatigue and restore normal contractility. However several studies (2; 1) have also demonstrated that during intense exercise, diaphragm performance may be limited by diaphragm blood flow. When the supply of energy falls below energy demands the force generating capacity of

the diaphragm fails resulting in respiratory failure. Smith-Blair and associates have demonstrated administration of dobutamine improves diaphragm blood flow with resultant increase in diaphragm contractility (15).

Diaphragm blood flow measurement: Until recently, two methods have been employed to measure diaphragm microcirculation blood flow. These included xenon clearance and radio-labeled or fluorescent microsphere techniques (14; 10). These methods however can provide only a few measurements in a given animal and can not be used continuously. Laser Doppler flowmetry (LDF) was originally designed to measure skin blood flow in humans. It allows a continuous recording of relative changes in local blood flow without interference with flow regulation which is a limitation in microsphere injections. In addition to measuring blood flow in the skin, the LDF has been used in a variety of other tissue including the brain, gastric mucosa, pig kidney, and human skin (5). It has not been extensively used to evaluate muscle blood flow microcirculation because of the limited laser beam penetration in thick, cylindrical tissues. However, the diaphragm is thin and flat and provides an ideal model to test the application of LDF in providing regional data on the microvascular perfusion of the diaphragm.

Pharmacological agents: Butoxamine. Butoxamine, a selective B_2-adrenergic antagonist that inhibits B_2-adrenoreceptors by competing with the catecholamines at the effector site. It has been used extensively in animal and tissue experiments (9; 17; 12) to characterize B_2- receptor involvement and identify B_2- receptors (9). Investigators have demonstrated that butoxamine produces selective blockade of vascular B_2-adrenoceptors while not stimulating cardiac B_1-adrenoreceptors at doses up to 3 mg/kg. The half-life of butoxamine is approximately 45 minutes in concentrations up to 3 mg/kg (4).

Salbutamol. Salbutamol was developed from the modification of norepinephrine and acts through highly selective B_2-adrenoreceptor receptor cell stimulation with effects on both smooth

and skeletal muscles. An increased heart rate is seen following administration of this drug but this may be due to a reflex response following relaxation of vascular smooth muscle resulting in vasodilatation rather than stimulation of B_1-adrenoreceptor receptor cells. It has minimal cardiac stimulant effect and does not cause hypertension. When administered intravenously, salubutamol circulates primarily as an unchanged drug due to avoidance of first pass metabolism. There is a dose dependent response when salbutamol is administered intravenously. The main route of excretion is by the kidneys with a half-life of approximately 4 hours (11).

Research Design and Methods: Research Design: A randomized experimental design with each animal serving as its own control will be used for this study. The experimental group will have three experimental periods: (a) Period 1, control, (b) Period 2- treatment in which loading dose of butoxamine (10mg/kg) will be infused intravenously followed by a maintenance dose of butoxamine at 10mcg/kg/min for 15 minutes. Following 15 minutes of infusion of butoxamine the infusion will be stopped. The animal will be allowed to rest for 1 ½ hours (two half lives of the drug). Period 3- Infusion of salbutamol will be given in intervals of 5mcg/kg/min and increasing after a 15 minute interval by 5 mcg/kg/min to a maximum of 20 mcg/kg/min. No recovery period will be used due to the extended half-life of the drugs being used. In addition, we will use one hamster as a sham by following the above periods 1,2, and 3, with the exception of not administering any medications in period 2.

Sample: Ten male Syrian Golden hamsters (150-180gms) will be used for this experiment. Animals will be housed at the Central Laboratory Animal Facility (CLAF) and be given lab chow and water ad libitum.

Experimental Protocol: During Period 1, baseline measurements will include diaphragm shortening (DS), intrathoracic pressure (ITP), laser doppler flow (LDF), heart rate (HR), respiratory rate (RR), arterial blood pressure (ABP), partial pressure of end tidal carbon dioxide ($P_{et}CO_2$), and

arterial blood gases (ABGs). In Period 2- DS, ITP, LDF1, HR, RR, and $P_{et}CO_2$ measurements will be measured following a 15 minutes stabilization period. During Period 3, after each 15 minute stabilization period for each concentration of salbutamol, the following will be measured: DS, ITP, LDF1, LDF2, HR, RR, and $P_{et}CO_2$. Following the last concentration of salbutamol, an ABG will be obtained following the 15 minute stabilization period.

Data analysis procedures: Data will be analyzed using a single sample repeated measures design to examine if differences exist between periods with respect to DS, ITP, LDF, ABP, ABG, HCT, &

An initial alpha level of 0.05 will be selected as the criterion for statistical significance. This level of significance will then be adjusted to an alpha level of .01 using an adjustment in the Bonferroni procedure to control for an overall type I error rate due to performing multiple analyses. To account for deviations in sphericity, the Greenhouse-Geisser Epsilon correction factor will be used.

Research Timetable	
January – March 2006	Conduct experiments and complete data collection
April – May 2006	Analysis and write up

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