Effect of EPA and HMB on Strength in ICU Patients

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Research Description

Form B: Research Description

1.Background.

Over the past 20 years there has been a dramatic growth in the numbers of patients requiring mechanical ventilation to sustain life in the United States. At most large medical centers, the number of patients needing more than transient mechanical ventilation for respiratory insufficiency has approximately tripled over the past 10 years. To deal with this burgeoning number of mechanically ventilated patients, stand-alone ventilator units have been created in the United States in most large cities, with literally hundreds of mechanically ventilated patients placed in such units in each of the large metropolitan areas in this country. In aggregate, we estimate that as many as 50,000 patients are on sustained mechanical ventilation at any given time in the United States. The total cost of caring for these patients now consumes approximately 6% of the yearly health care budget, or upwards of 1% of the United States GNP.

We have recently completed a study at the University of Kentucky that indicates that a major process contributing to respiratory failure in critically ill patients is respiratory muscle weakness. This work found that the average patient in the UK ICU has a diaphragm pressure generation (PDI) in response to phrenic magnetic twitch stimulation of only 8 $\pm$ 3 cm H2O, compared to an accepted normal value of 30 cm H2O for healthy adults. Moreover, we found that 70% of patients have twitch PDI measurements that are less than a third of normal. These data suggest, moreover, that severe respiratory muscle weakness contributes to respiratory failure in mechanically ventilated patients, with the weakest patients taking the longest to wean from mechanical ventilation and having the highest mortality.

Unfortunately, there is currently no specific defined treatment to prevent or reverse respiratory muscle weakness in mechanically ventilated patients. Theoretically such a treatment may improve outcomes in these patients, reducing the time required to wean from mechanical ventilation, shortening hospital duration, and potentially improving mortality. While no specific treatment is available for patients with respiratory muscle weakness, animal studies indicate that there are defined cellular pathways (calpain, caspase, free radicals, the proteasomal pathway) that contribute to diaphragm weakness in response to the clinical stresses placed on critically ill patients (i.e. inactivity due to mechanical ventilation, inflammation secondary to infection, hyperglycemia). Moreover, these pathways can be blocked and diaphragm dysfunction prevented in these animal models of disease by administration of either eicosapentaenoic acid (EPA, a caspase and calpain inhibitor)) or hydroxymethylbutyrate HMB (a caspase and PKR inhibitor). Both these agents, EPA and HMB, have been safely administered to many patients in previous studies (EPA to prevent vascular disease, HMB to improve muscle function in cancer patients). Neither drug has been reported to cause significant side effects at the dosages chosen. Both are over the counter supplements that can be obtained without prescription in drug stores, health food stores and over the internet.

Based on this information, we postulated that it may be possible to prevent the development of diaphragm and limb muscle weakness in critically ill, mechanically

ventilated patients by early administration of either EPA or HMB. We recently completed an IRB approved pilot study examining the effects of EPA and HMB on diaphragm strength in a small group of mechanically ventilated MICU patients. This study found that both agents induced increases in diaphragm strength and that administration of HMB reduced the duration required to wean patients from mechanical ventilation. These pilot data were used to submit a grant application to the NIH, entitled **"Treatment of Skeletal Muscle Dysfunction in ICU Patients",** which was subsequently funded.

2. Objectives.

The objective for this study is to determine if administration of either EPA or HMB can prevent or reverse the development of respiratory muscle weakness in critically ill, mechanically ventilated patients. We plan to randomize patients accepted into this protocol to administration of either a control (saline enteral control solution), EPA administration, HMB, or the combination of EPA +HMB. Drugs will be administered for 10 days and measurements of muscle strength (diaphragm and quadriceps), muscle size (diaphragm and quadriceps thickness by ultrasound), and muscle signaling protein profile (obtained using vastus lateralis muscle biopsy specimens) will be performed immediately before and immediately after the period of drug administration (days 1 and 11). An additional assessment of muscle strength, muscle size and signaling protein profile will be made at 10 days after completion of the drug administration regimen (i.e. on day 21). As part of the study, patients will be weaned from mechanical ventilation using the University of Kentucky ventilator separation protocol, the nutritional regimen will be reviewed with the MICU nutritionists to ensure it conforms to study requirements, and it is expected that patients will receive standard MICU mobilization therapy by physical therapy.

We will also perform a chart review and assess ventilator mechanics (respiratory system static compliance and inspiratory airway resistance) at the time of strength assessment. We would expect that mean diaphragm and quadriceps muscle strength and size measurements will be similar for four groups immediately before initiation of drug administration. Our hypothesis will be supported if post drug administration diaphragm and limb muscle strength are higher for patients receiving EPA , HMB and/or the combination of EPA+HMB when compared the control group receiving no active drug. We will also determine if administration of these agents shortens the time required to wean patients from mechanical ventilation.

3. Study Design.

The basic study design is to:

(a) measure magnetic stimulated diaphragm Pdi twitch and quadriceps twitch strength, measure diaphragm and quadriceps thickness using ultrasonography, obtain a vastus lateralis muscle biopsy, determine lung mechanics (respiratory system compliance, determine airway resistance), and perform a chart review,

(b) randomize patients to treatment with either:

Placebo this will be given as two tablespoons of salt water solution, 1.5 grams of amino acids and 0.2 teaspoons of corn oil every 12 hours,

Eicosapentaenoic acid (EPA); this will be given as two tablespoons of salt water, 1.5 grams of amino acids and 1000 mg EPA every 12 hours,

Hydroxymethylbutyrate (HMB); this will be given as two tablespoons of salt water solution, 1500 mg HMB and 0.4 teaspoons of corn oil every 12 hours,

HMB and EPA; two tablespoons of salt water, 1500 mg HMB and 1000 mg EPA every 12 hours.

(c) continue drugs for 10 days then

(d) on day 11, remeasure magnetic stimulated diaphragm Pdi twitch and quadriceps twitch strength, measure diaphragm and quadriceps thickness using ultrasonography, obtain a vastus lateralis muscle biopsy, and determine lung mechanics (respiratory system compliance, determine airway resistance), and

(e) on day 21, remeasure magnetic stimulated diaphragm Pdi twitch and quadriceps twitch strength, measure diaphragm and quadriceps thickness using ultrasonography, and determine lung mechanics (respiratory system compliance, determine airway resistance) if the patient is still requiring mechanical ventilation.

In preliminary work we have verified that HMB easily goes into solution when placed in saline. In addition, EPA goes into solution easily when placed in a mixture of oil and saline (in a 1:3 ratio). We will plan to administer the agents at 7 AM and 7 PM whenever possible so that the administration of these agents does not interfere with dosing of patient medications. The IDS will perform both blinding and randomization of the agents provided. The IDS will perform randomization using an online computer program (www. Randomization.com). Note that HMB has no side effects at any dosage. At higher doses than the doses to be used in the current study, EPA can interfere with clotting and potentiate the effects of aspirin and Coumadin.

For each chart review we will obtain the following information: age, sex, diagnoses, medications, reason for institution of mechanical ventilation, vital signs, bedside parameters of mechanical ventilation use (including mode of ventilation, duration of ventilation, level of oxygen, breath volume and rate, % triggered breaths), most recent arterial blood gas values, chest radiograph readings, recorded assessments of mental status.

4. <u>Study Population.</u>

Adult patients requiring mechanical ventilation for respiratory failure for more than 24 hours in one of the University of Kentucky adult ICU's will be asked to participate. An additional inclusion criterion is that the attending physician caring for the patient must anticipate that the patient is likely to remain on mechanical ventilation for more than 24

hours. We will include patients regardless of gender, race, or adult age. It is hoped that sufficient minorities and women will be studied so that the subject population is representative of the general patient population, but we will be somewhat constrained by the numbers of available patients and the day to day makeup of the patient population in the UK ICU's. Inclusion of minorities and women will make the study results more generally applicable.

Patients will be excluded: (a) if the physician caring for the patient determines that the patient is too unstable to tolerate these measurements, (b) if the patient requires high dose pressors (more than 15 mcg/min of norepinephrine or more than 15 mg/kg/min of dopamine), (c) if the patient requires more than 80% FiO2 or more than 15 cm H2O of PEEP, (d) if the patient has a cardiac pacemaker or implanted defibrillator, (e) if the patient has received neuromuscular blocking agents within the 48 hours preceding testing, (f) if the patient has a history of a preexisting neuromuscular disease, (g) if the patient has a history of recent variceal bleeding, (h) if the patient has profound and uncorrectable hypokalemia (less than 2.5) or hypophosphatemia (less than 1.0), (i) if the patient is a pregnant female, (j) if the patient is a prisoner, (k) if the patient is institutionalized, (l) if the patient is allergic to fish, (m) if the patient cannot receive enteral medications, (n) if it is thought that the patient is terminal and will have care withdrawn within days, (o) in addition, if a given patient has a coagulopathy at the time of enrollment into the study (defined as an elevation of the INR above 1.5, an aPTT 50% or more above the normal range, or a platelet count less than 60,000), we will not perform muscle biopsies on the patient but will collect the other data planned.

5. Subject Recruitment.

Our goals are to recruit and randomize 80 patients into the study (2 patients/month). If patients drop out of the study during initial measurements (because of unwillingness to complete the protocol or for technical issues, e.g. inability to attain supramaximal conditions during magnetic stimulation) we will recruit additional replacement subjects so that the desired randomized numbers (80 total) are achieved. We believe this is a realistic timetable. Our institution has three MICU attending services and currently has 40-50 patients/day on these services, with the great majority (i.e. 30-40 at any given time) on mechanical ventilators. When performing our pilot studies we found that is possible to recruit as many as 3 patients/week from our MICU population. As a result the recruitment requirements of the current study are a fraction of the number of patients available from the MICU population at the University of Kentucky. It should also be noted that, the families that we approached to obtain pilot data for this proposal agreed enthusiastically to participate in the study. As a result, based on our recent track record, we believe we can easily recruit the patients required to complete these protocols.

The PI of this study will meet 3-5 times a week with the MICU attendings at the University of Kentucky to identify patients who would be suitable candidates for this study based on the study inclusion and exclusion criteria. The MICU attending will then approach the patient and the patient's LAR (the legally appointed person that can provide consent) to determine if the patient agrees to allow the PI to discuss the study

with them. The PI will then approach the family and review the study with them and ask if they wish to participate. The consent form will be reviewed with the patient and their LAR detailing the risks associated with the planned measurements. If the patient and LAR agree, the consent form will be signed and the investigator will obtain the required equipment to assess quadriceps and diaphragm twitch measurements. Measurements will be made at the bedside, in the patient's room. No form of advertisement will be used for this study.

6. Informed Consent.

After identification of a potential study subject during the interaction between the PI and the MICU attending, the subject and their LAR will be approached in the MICU within the next 1-3 hours. If they decide to be included in the study, the consent form will be reviewed with them immediately. The PIs (Dr. Gerald Supinski and Dr. Leigh Ann Callahan) will obtain all informed consents for the study. To eliminate coercion, all LARs and study participants will be told that refusal to participate will in no way influence their care while at the University of Kentucky. Consent will be documented by having both the patient and the LAR sign the consent form. Because most ICU patients receive some form of sedation, it is the intent of the PI to always inform the LAR as well as the patient and always have the LAR sign the consent form. Under certain circumstances the patient may be so disabled that signage of the consent form is not possible (e.g. both hands are instrumented with IV's and pressure transducers) or may be very sedated. In the event of physical inability of the patient to sign the consent form, the LAR alone will sign the form.

7. Research Procedures.

It will first be verified that the patient does not meet any exclusion criteria and that informed consent has been obtained.

Diaphragm Strength: The PI will then place gastric and esophageal balloon tipped catheters to allow measurement of transdiaphragmatic pressure (Pdi). We will employ sterile single use commercially available balloon tipped catheters (from Ackred Medical, NJ) which have been manufactured specifically for this use and are in widespread usage in the US and other countries for this application. Each catheter will be inserted via the nose after topical lidocaine application (1 cc of 1% Lidocaine gel applied to the outside of each catheter) and directed via the esophagus. One catheter so placed will be inserted until the tip lies in the stomach while the other will be left in the esophagus. Correct placement will be verified by connecting the catheters to Validyne pressure transducers and assessing pressure waveforms during various maneuvers. The correctly placed gastric catheter will generate a positive pressure during spontaneous breathing efforts, mechanical breaths, and with gentle pressure over the stomach. The esophageal catheter should generate negative pressures during spontaneous breathing efforts, positive pressures during mechanical breaths, and little or no pressure during gentle pressure over the stomach. The pressure signals from the two catheters will "added" (i.e. the absolute swings in gastric and esophageal pressure during stimulation will be summed) to obtain transdiaphragmatic pressure (Pdi). Pressure signals will be recorded continuously using a flat bed strip recorder. Once pressure signals are being recorded, a sterile pneumatic occlusion valve will be placed in the ventilator circuit

between the endotracheal tube and the ventilator tubing y connector. Subsequently, bilateral magnetic coils will be placed over the phrenic nerves on the anterior neck. The occlusion valve will be activated between spontaneous breaths for approximately half a second and, during the occlusion, bilateral magnetic pulses will be applied to the phrenic nerves to generate a diaphragmatic twitch. Twitches will be repeated to obtain three reproducible maximal twitches. This assessment will take approximately 5 minutes. Once these measurements are completed, the esophageal and gastric balloons will be withdrawn.

Quadriceps Strength: For this assessment, we will place a quadriceps board (Bailey Manufacturing) under the left leg, positioned so that the knee lies immediately over the apex of the apparatus. We will then strap a force transducer (Interface, Model SSM-AJ) over the lower leg, positioned at one-fourth the distance between the lateral malleolus of the ankle and the knee. We will first assess twitch force with the apparatus set at the fourth “peg” position, corresponding to a 90o angle between the thigh and lower leg (we have found this corresponds to the optimal angle for quadriceps force generation in the majority of subjects). We will then adjust magnetic field strength during femoral nerve stimulation to ensure optimization of twitch force (i.e. usually at 85% of the maximum Magstim magnetic field strength). Five consecutive twitches will be performed at this setting. Subsequently, to verify the 90o knee angulation setting represents the optimal preload, twitches will be repeated at knee angle settings above and below 90o (i.e. at “peg” settings of 2 and 6). The average of the three best twitch forces generated at the optimal knee angle with supramaximal field strength will be averaged and this measure will be reported as the quadriceps twitch force.

Muscle Size: Diaphragm thickness will be assessed using a 2D ultrasound unit (Sonosound). For this determination an abdominal ultrasound transducer will be placed over the right eighth-ninth intercostal space in the mid axillary line. The probe will be angled to identify the diaphragm which appears as a three layered structure just above the liver (see figure 8); the echogenic outer lines of this structure represent the peritoneal and diaphragmatic pleural layers with the inner layer representing muscle. With spontaneous inspiration, this portion of the diaphragm thickens, and then thins at end expiration. Images will be taken at end expiration and diaphragm thickness measured with the sonoprobe caliper utility. In our experience it is easier to identify the diaphragm and measure its thickness on the right side than on the left, and it is because of this that we have chosen to assess right diaphragm thickness. Our normal controls have had end expiratory diaphragm thicknesses between 0.40 and 0.55 cm. We will employ a recently described approach to assess quadriceps size using ultrasound. The quadriceps is composed of four muscles, so this technique measures the thickness of each of these components of the quadriceps (vastus lateralis (VL), vastus medialis (VM), vastus intermedius (VIM), rectus femoris (RF), determined as the distance from the adipose tissue-muscle interface to the intermuscular interface for each muscle. The measurements for the VM will be taken at 30% and those for the VL, VIM, and RF at 50% of the distance between the lateral condyle of the femur and the greater trochanter.

Muscle Biopsy: We plan to biopsy the vastus lateralis leg muscle to obtain tissue for assessment of signaling pathways and single fiber force-pCa assessments. Biopsy of the vastus lateralis is a widely used, conventionally accepted approach to obtaining

skeletal muscle for analysis in patients, this procedure is very safe, and one of the investigators (Dr. Callahan) has significant experience in obtaining tissue by this means. For all these reasons, we plan to obtain vastus lateralis biopsies whenever possible in recruited patients (if a clotting disorder is present but a patient otherwise qualifies for study, we will include the patient but omit the muscle biopsy). Patients will be on continuous tube feedings (we believe it would be incompatible for ethical ICU care to fast these critically ill patients before obtaining biopsies). Patients will be at bed rest over night prior to performance of all biopsies and, more specifically, no patient will be exercised over night in the morning before biopsies are obtained. For the biopsy procedure, the skin will be sterilely prepped over the muscle, the skin infiltrated with lidocaine, a small skin incision made, and a Bergstrom needle advanced into the muscle. Suction will be applied to the Bergstrom needle and two-three biopsies taken (total anticipated muscle yield of 200 mg). The needle will be withdrawn, a portion of the biopsy will be dissected free of adipose tissue and frozen in liquid nitrogen for latter assessment of signaling pathway protein levels. The remaining tissue will be processed for single fiber force-pCa determinations and fiber type determination using histochemistry.following the biopsy, pressure will be applied at the biopsy site and an occlusive dressing placed. The investigators will return to examine the biopsy site for seven days to confirm adequate site healing.

Ventilator Mechanics: The mechanical ventilator will then be viewed and ventilator settings (tidal volume, mode of ventilation, oxygen concentration, minute ventilation, breathing frequency, PEEP level) recorded. Respiratory system static compliance, airway resistance, and autoPEEP levels will also be determined and recorded used the Siemens ventilator built in diagnostic module.

Clinical Parameters: These parameters (age, gender, diagnoses, diagnosis of infection [positive cultures or clinical diagnosis of sepsis], antibiotic usage, glucose levels, albumin levels, BUN, SOFA scores, time on mechanical ventilation before testing, use of corticosteroids, vital signs, reason for institution of mechanical ventilation, mode of mechanical ventilation, duration of ventilation, FiO2 level, breath volume and rate, % triggered breaths, most recent arterial blood gas values, chest radiograph readings) will be obtained from the medical record using values collected as closely as possible to the time at which physiological data measurements are made. To provide an assessment of pre-hospital function, family members will be asked four questions: (a) how far could the patient ambulate, (b) how many flights of steps could the patient go up, (c) which activities of daily living (dressing, carrying objects, performing daily grooming tasks), if any, were impaired, and (d) did the patient have difficulty standing from a sitting position or lifting objects over their head.

Hospital Outcomes: After recruitment into studies, electronic hospital records will be reviewed at weekly intervals to determine the time from the point of initial study measurements to successful weaning from mechanical ventilation (defined as remaining off all mechanical ventilation for more than 4 days). Alternative outcomes that will be recorded, for patients that are not weaned from mechanical ventilation, include transfer to a chronic facility for long term weaning from mechanical ventilation (i.e. an LTAC) or death. Deaths will be further subclassified as death due to withdrawal of care and death due to progression of disease. We will also record total ICU stay, total days of hospital

stay, and incidence of recurrent respiratory failure, defined as a requirement for reintubation either during the initial hospitalization or at any time out to day 60 after entry into the study.

Ventilator Separation Protocol: All patients will be weaned using the University of Kentucky Ventilator Separation Protocol. This protocol is currently employed in all University of Kentucky adult intensive care units and provides a standardized approach to weaning patients from mechanical ventilation. Every morning mechanically ventilated patients are evaluated for potential weaning trial employment. Inclusion criteria for a spontaneous breathing trial (SBT) includes evidence that the patient is: (a) awake, (b) has a gag reflex, (c) has a cough reflex, (d) has a RASS scale of -1 to 0, (e) has a FiO_2 ≤50% and PEEP applied ≤ 6 H_2O, (f) has a pH ≥7.35, (g) has a MAP of ≥ 65 mm Hg, (h) is off pressor drips (e.g. epinephrine) save for doses of dopamine ≤ 5 mcg/kg/min or dobutamine ≤ 5 mcg/kg/min, and (i) is not on an intra-aortic balloon pump. Exclusion criteria include increased intracranial pressure, use of paralytics, active seizures, excessive agitation and active myocardial ischemia. If the patient meets all inclusion criteria and has no exclusion criteria, sedation will be reduced and the patient will receive a spontaneous breathing trial (SBT) for 1 hour, with an ABG drawn at the end of the trial. During the trial patients will be monitored serially for evidence of weaning success or failure (i.e. observed at the 5, 15, 30, 45 and 60 minute time points into the trial).Trials will be stopped if any of the following events occur: (a) oxygen saturation falls below 90% for more than three minutes, (b) the heart rate increases above 130 or falls below 60, (c) systolic blood pressure falls below 90 or increases above 180, (d) the mixed venous oxygen saturation falls below 60 mm Hg (if monitored), (e) the respiratory rate increases above 35 or the RSBI increases above 100, (f) the patient develops chest pain or a new dysrhythmia, (g) the patient's mentation worsens, or (h) the patient develops diaphoresis, distress or excessive anxiety. Blood gases will be examined at the end of trials and trials will be judged a success if, in addition to passing the criteria in the preceding sentence, the final paO_2 is greater than 60 mm Hg and there is no respiratory acidosis. If the patient achieves a successful SBT, the attending will be asked for an extubation order. If the trial fails, the patient will be placed on their previous ventilator mode and settings. If trial failure is associated with evidence of excessive sedation, sedation levels will be reduced in preparation for the next day trial.

Nutrition Protocol: At study entry the nutritional plan developed by the MICU nutrition support service will be reviewed with the nutritional consultant for the patient. In general, the following protocol will be used by this service to provide tube feeds to our patients (prepared with input from Barbara Magnuson, head of the MICU nutritional team at the University of Kentucky):

(1) First, basal energy expenditure (i.e. caloric requirements) will be estimated based on the Harris- Benedict equations:

BEE Men = 66 + (13.7 x Wt kg) + (5.0 x Ht cm) – (6.8 x Age yrs)

BEE Women = 665 + (9.6 x Wt kg) + (1.7 x Ht cm) – (4.7 x Age yrs)

(2) Second, adjustment will be made for obesity if >125% ideal body weight by adding 25% of the difference between BEE for actual and ideal body weight to the BEE for ideal body weight.

(3) Third, a multiplier will be used to adjust caloric requirements to adjust for severity of illness; 1.2 for stroke, 1.3 for pneumonia or ARDS

(4) Fourth, we will estimate protein needs for unstressed patients as 0.8-1.0 gm/kg, for mild stress as 1.0-1,2 gm/kg, for moderate stress as 1.2-1.5 gm/kg, for infection or severe stress as 1.3-1.6 gm/kg; actual body weight will be used unless >125% of ideal body weight in which case adjusted body weight will be used, as calculated in (2)

(5) Fifth, additional adjustments in nutrition will be made for patients with renal or liver failure based on the University of Kentucky Adult Nutrition Support Handbook

(6) Sixth, an enteral feeding product is chosen; for the present study a good formulation is Jevity 1.2 which is composed of 18.5% protein calories, 29% fat calories, and 52.5% carbohydrate calories. For Jevity 1.2, the total calories required per day as calculated in (3) will be divided by 1.2 cal/ml to derive the volume of Jevity 1.2 to be provided over 24 hours. Of note, patients receiving Juven will not be enrolled in the study as this product contains HMB (It is not the current practice of MICU physicians at UK to use this formula). We will also not study any patients fed any other tube feed formulations containing eiter EPA or HMB.

(7) Once the Jevity 1.2 volume is determined, the protein provided by this delivery will be calculated; additional protein to reach the daily protein needs of the patient will be provided by adding Beneprotein supplement to bring the total protein delivery to that calculated in (4).

(8) Enteral access will be established, if not already present, as per UK Enteral Access Guidelines

(9) The volume of enteral feeding will be administered as a continuous feed delivered by a pump over 24 hours.

8. Resources. The PI has studied various aspects of respiratory muscle function and dysfunction over a 25 year period. During this time, the PI has published approximately 100 peer reviewed papers on original research related to respiratory muscle and other critical care topics, as well as 150 abstract presentations at national meetings. The PI has given state-of-the art presentations on assessment of Pimax at the national American Thoracic Society meeting and is a co-author of the ATS-ERS International statement on measurement of respiratory muscle function. The PI has published several papers employing assessment of both Pimax and transdiaphragmatic pressure measurements. In addition, the PI has practiced critical care medicine for 25 years, measuring Pimax in approximately 2000 patients over that time span. In addition, in both northern Ohio and Western New York the PI was the regional resource providing measurement of transdiaphragmatic pressure when this was required clinically.

The PI is currently funded by an NIH grants as PI and also receives support as a co-investigator from another NIH grant held by Dr. Callahan. The PI's laboratory is equipped with a cart containing all the apparati required to obtain transdiaphragmatic pressure readings, including Ackrad balloon tipped catheters, Validyne differential pressure transducers, Validyne preamplifier racks (3 preamps/rack, two racks), a pressure manometer for calibration, and a two channel recorder. This cart is mobile and can be moved to the ICU rooms when needed to assess Pdi-twitch. The cart is

equipped with sterile wipes that will be used to disinfect all exposed surfaces before and after entrance and exit from patient rooms. The cart is also equipped with two MagStim 200 magnetic stimulators equipped with dual figure of eight coils for phrenic nerve stimulation. This device is FDA approved and is used regularly for the purpose of performing Pdi-twitch assessment in the USA and around the world.

Ackrod balloon tipped catheters and t-pieces will be disposed of after use and new catheters/tpieces employed for every set of measurements. The ventilator circuit pneumatic occlusion valves will be sterilized by autoclaving prior to use.

9. Potential Risks

The magnetic twitch assessment procedures described in the protocol have been used extensively in both the US and England. We have performed this assessment many times in our ICU population over the past year both to recruit patients into other studies and to perform diagnostic studies on ICU patients for routine management issues (i.e. to detect diaphragm paralysis, to assess the response to treatment for myasthenia and other neuromuscular diseases, to assess patients with respiratory failure of unclear etiology). We have not had a single complication from our use of magnetic stimulation per se or from placement of esophageal and gastric balloon tipped catheters for pressure assessment. In addition, the PI recently visited Dr. Polkey and Dr. Moxham's laboratories in England and spoke directly with them regarding their experience with the magnetic twitch-Pdi technique. They have not observed a serious complication from this procedure and reported only minor temporary discomfort from the delivery of the magnetic stimulus or from placement of the esophageal and gastric balloons. There is no reported long term risk reported in response to single stimulus excitation of peripheral nerves (such as the phrenic nerve) using a magnetic stimulating device. The magnetic stimulator to be employed (Magstim 200) is commonly used for the application proposed and is FDA approved for stimulation of peripheral nerves.

We extensively searched the Internet and other sources and, to our knowledge, hydroxymethylbutyrate (HMB) has no known side effects at any dosage. This agent is a biopharmaceutical, a normal breakdown product of leucine, and is present in small quantities in all human beings. The other agent to be employed in the present study, eicosapentaenoic acid (EPA) has only been reported to produce minor side effects (occasional nausea, diarrhea, heartburn, skin rash, itching) at the dosage to be employed in the current study. At high doses this agent may potentiate the effects of Coumadin or interfere with platelet function; for this reason we will not use doses higher than the one included in the study protocol. Both drugs are available as over the counter medicines and are not subject to FDA regulation.

Risks associated with biopsy of the vastus lateralis muscle include bleeding into the biopsy site and potential infection at the site. There should be no risks associated with measurement of muscle size via ultrasound or chart review to assess clinical parameters. As indicated in the above paragraphs most of procedures included in this study are extremely safe, with no possibility of patient injury due to ultrasound measurement, magnetic stimulation or HMB administration. In addition, it is difficult to imagine that the low dose of EPA to be used will produce significant injury. The only component of the experimental plan that could potentially cause patient injury is the

vastus lateralis biopsy. If the investigators detect problems as a result of the biopsy, or if the family or professionals caring for the patient complain of biopsy complications or report other possible complications that result from these studies, we will stop experimentation, and the patient will be withdrawn from the study.

10. Safety Precautions.

To minimize the risk of bleeding from balloon placement, patients with variceal bleeds will be excluded from this study. Because assessment of PdiTw involves transient removal from mechanical ventilation, patients requiring high levels of PEEP, high levels of oxygen or in severe shock will be excluded from study. As indicated in above paragraphs, these procedures have been extensively employed in London, UK to evaluate patients in both outpatient and ICU settings and the investigators there have not observed complications with either balloon placement or magnetic stimulation. In fact there are no reported adverse effects using magnetic stimulation to assess peripheral nerves except for an isolated report of interference with pacemaker function in one patient. For this reason, we will also exclude patients with pacemakers from this study. As per standard ICU protocols at UK, during all measurements all patients will be connected to invasive or non-invasive blood pressure transducers, EKG monitors, and pulse oximetry monitors, providing a continuous assessment of patient pulse rate, blood pressure, and oxygen saturation during assessments. If desaturation to less than 90%, a raise in heart rate by more than 20 beats, a rise in mean blood pressure more than 10 torr or a fall in mean blood pressure of more than 10 torr is noted, measurements will stop. The PI will return at 24 hours after each study to the patient's bedside to ascertain if any untoward events have occurred and to elicit feedback regarding any concerns that the patient and/or their family and/or the nursing staff caring for the patient may have If problems are encountered they will be reported to the IRB and data monitoring board.

To minimize the risk that the patient's privacy would be compromised, all data will be placed into a file at the conclusion of the bedside study and this file will be placed into a locked file cabinet in the office of the PI. Each file will be assigned a research number, and all data abstracted from the file of an individual subject will be referred to in later data analysis by this patient file number with no reference to any identifiable patient characteristics (e.g. name, SS number, hospital number). The mean data for the study will also be placed in a mean data file and this information also kept in the locked file drawer in the PI's office.

11. Benefit vs. Risk.

Based on our previous experience and the reported experiences of others, we believe the major stress that will be felt by subjects will be the discomfort associated with balloon-tipped catheter placement into the esophagus as well as the momentary sensation associated with magnetic stimulation. The sensation associated with magnetic stimulation is not painful but is more like a "jolt" related to the sudden involuntary contraction of the muscles being stimulated distal to the nerve. We do not believe these techniques pose a significant risk to patients, in that no long term adverse consequences should occur providing the exclusion criteria used for this study are rigorously followed.

Neither of the drugs to be used (HMB or EPA) has been reported to cause side effects at the dosages chosen. In fact, we did not find report of any side effects from these doses of HMB. EPA has been reported to cause minor side effects in occasional patients (nausea; diarrhea, heartburn, skin rash, itching, and joint, back, and muscle pain). Both agents are over the counter agents that are sold as health food supplements and are available without prescription. Neither is an FDA regulated substance.

Patients randomized to drug treatment (HMB and/or EPA) may well be stronger as a result of this treatment and may well have a shortened ICU stay, a lower mortality, and less long term morbidity (i.e. better ambulation, less dyspnea).There are, however, important potential benefits for the patients taking part in this study. To date, our work in the ICU indicates that the average twitch transdiaphragmatic pressure generated by ICU patients is only 23% of the value seen in normal healthy adults. In animal studies, both HMB and EPA have been shown to preserve diaphragm strength in models of critical illness. If these drugs also prove effective in preserving or improving diaphragm strength in ICU patients, the patients randomized to receive these agents may be able to recover from respiratory failure faster and be weaned from mechanical ventilation sooner than control patients receiving control solutions.

12. Available Alternative Treatments.

Currently, there is no defined drug treatment to prevent or reverse the development of weakness in critically ill patients. As a result, there is no acceptable alternative to the clinical trial drugs (HMB, EPA) planned for these studies. While one might think that androgens would be an acceptable alternative, a recent study found these agents worsen lung function and actually had deleterious effects on outcomes when tested in surgical ICU patients. Currently there are no drugs that have been identified that can improve respiratory muscle strength in critically ill patients. As a result, there are no alternative treatments to replace the agents being studied in the current project.

13. Research Materials, Records, Privacy.

All data collected from the study (i.e. tracings of pressure measurements, demographic/clinical data obtained from chart review, the survey form filled out by the attending physician) will be placed in a folder which will be stored in a locked filing cabinet in the office of the principal investigator in MN616. None of this information will be shared with other investigators. All folders will be given a study number and further analysis of mean data obtained from patient study folders will be carried out with only the patient study number as identification. Folders containing mean data will also be stored in the locked filing cabinet in MN616.

14. Confidentiality.

For publication, no individual identifying features of the patients will be included in abstracts and papers generated using the data collected in this study. As indicated in section 13, all records will be kept in a locked file cabinet in the PI's office. The records for this study will be kept until seven years after the data is published, in keeping with current NIH recommendations on data archiving. The data will be kept in the locked file cabinet in MN616 at least until the data is published and then transferred to a second locked archival filing cabinet in the PI's office. At seven years after publication all data

will be destroyed by shredding the documents. All data collected from the study (e.g. pressure, force, and muscle size measurements, demographic/clinical data obtained from chart review) for a given patient will be placed in a folder which will be stored in a locked filing cabinet in the office of the principal investigator in MN616. All folders will be given a study number and further analysis of mean data obtained from patient study folders will be carried out with only the patient study number as identification. Folders containing mean data will also be stored in a locked filing cabinet in MN616 of Chandler Medical Center, University of Kentucky. For publication, no individual identifying features of the patients will be included in abstracts and papers generated using the data collected in this study.

15. Payment.

No subject will be paid for participation in the study.

16. Costs to Subjects.

There will be no cost to subjects for participation in the study.

17A. Data Monitoring: Data Safety Monitoring Board

A detailed description of our Data Safety Monitoring Plan is attached. In brief, to monitor the long term conduct of the study, including monitoring of adverse effects, we will employ the study monitoring board provided by the CCTS at the University of Kentucky. This board will include the chair, Lisa Tannock, MD, Linda Rice, RN, and other current members of the DSMB.

Monitoring for adverse events will be conducted in real-time by the study investigators and study coordinator. Risks involved with this study are considered greater than minimal risk. For this reason, we will utilize the standing independent Data Safety Monitoring Board (DSMB) as chartered by the University of Kentucky Center for Clinical and Translational Science (CCTS) to monitor the safety of the study.

The DSMB will meet three times a year and will review subject recruitment, adverse effects, side effects, laboratory results, withdrawals, protocol violations, and inclusion/exclusion criteria. More frequent meetings will take place if necessary. The board will compile a report for each meeting and present their findings to the PI.

We will also have the DSMB provide the recommendations they send to us to the NHLBI. We will also provide the NIHLBI with the meeting dates of the DSMB. The NIH will also be notified of all adverse effects and will have our SAE form send to her along with the safety officer, the IRB and the DSMB.

17B. Safety Monitoring

During the execution of this protocol the nurse assigned to the patient will be asked to be present in the room to provide an ongoing assessment of patient vital signs. As per standard ICU protocols at UK, during all measurements all patients will be connected to invasive or non-invasive blood pressure transducers, EKG monitors, and pulse oximetry monitors, providing a continuous assessment of patient pulse rate, blood pressure, and oxygen saturation. If desaturation to less than 90%, a raise in heart rate by more than 20 beats, a rise in mean blood pressure more than 10 torr or a fall in mean blood pressure of more than 10 torr is noted, measurements will stop and the patient will be

returned to mechanical ventilation. As per standard ICU protocols at UK, during all measurements all patients will be connected to invasive or non-invasive blood pressure transducers, EKG monitors, and pulse oximetry monitors, providing a continuous assessment of patient pulse rate, blood pressure, and oxygen saturation during assessments. If desaturation to less than 90%, a raise in heart rate by more than 20 beats, a rise in mean blood pressure more than 10 torr or a fall in mean blood pressure of more than 10 torr is noted, measurements will stop. The PI will return at 24 hours after each study to the patient's bedside to ascertain if any untoward events have occurred and to elicit feedback regarding any concerns that the patient and/or their family and/or the nursing staff caring for the patient may have If problems are encountered they will be reported to the IRB and data monitoring board. The PI or Dr. Callahan will also return to inspect the muscle biopsy site daily for seven days to confirm adequate healing at the site.

The PI will provide the nurse with his cell phone number at the completion of each data collection with instructions to call the PI if any untoward events occur within the following 24 hours. In addition, the PI will return at 24 hours after each study to the patient's bedside to ascertain if any untoward events have occurred and to elicit feedback regarding any concerns that the patient and/or their family and/or the nursing staff caring for the patient may have. If problems are encountered they will be reported to both the monitoring board described in the preceding section and to the IRB board by the PI.

18. Subject Complaints.

After obtaining consent, the patient and his family will be given a sheet providing the office number, paging number, and cell phone for the PI and instructions on how to contact the PI. We will provide the patient and his family any information obtained from the Pimax and Pdi-twitch measurements, and will provide them with information as to whether the measured numbers are normal or reduced. If any complaints are registered by a subject these will be recorded and placed in the patient's study file. This information will be handled in a confidential matter (see above).

19.Research With Non-English Speaking or Foreign Cultures:

We do not plan to study patients that, because of language barriers, cannot understand English. This study is too complicated to be dealt with adequately through interpreters.

20.HIV Research:

This is not applicable.

21. FDA Regulated Research:

This is not applicable.

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