2005-08-30

In vivo characterization of respiratory forces on the sternal midline following median sternotomy

Shruti Pai
Worcester Polytechnic Institute

In vivo characterization of respiratory forces on the sternal midline following median sternotomy

A Thesis Report

Submitted to the Faculty of the

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Master of Science

by

Shruti Pai

August 30, 2005

Approved:

Kristen L. Billiar, PhD, Thesis Advisor

George Pins, PhD, Committee Member

Raymond Dunn, MD, Committee Member

Acknowledgments

I would like to thank the many friends, family, and colleagues who made the completion of this project possible. I am especially grateful to the following people:

Dr. Nicola Francalancia, Dr. Russell Babbitt, Heather Strom, Suzanne Wheeler, and Timothy Roth of the University of Massachusetts Medical School Department of Surgery for their assistance with the surgeries.

Dr. Maggie Delano, Dr. Denice Godfrey, Dr. Paul Bergman, Sheila Donahue, and Robin Knox of the University of Massachusetts Medical School Department of Animal Medicine for accommodating our special needs without hesitation.

Dr. A. Kumar and Karen Baldwin of Tufts University for teaching me their large animal embalming procedure.

Professor Jason Wilbur for his statistical advice and Steve Derosier, Jim Johnston, Will Johnston, Russ Dresher, and Helena Zec of Worcester Polytechnic Institute for their assistance with building the hardware and software components of my instrumentation.

Professor George D. Pins and Dr. Raymond M. Dunn for their invaluable advice, patience, and encouragement in their role as committee members.

Professor Kristen L. Billiar for being an exceptional mentor, motivator, and teacher and without whom I would never have summoned the courage to start this project or acquired the knowledge to finish it.

This work was supported in part by Johnson & Johnson Wound Management at the University of Massachusetts Department of Surgery.

Abstract

The development and clinical adoption of more effective fixation devices for re-approximating and immobilizing the sternum after open-heart surgery to enable bony healing has been limited, in part, by the lack of *in vitro* test methods used to evaluate these devices which precisely emulate *in vivo* loading of the sternum. The present study is an initial effort to determine the loading parameters necessary to improve current *in vitro* and numerical test methods by characterizing the direction, magnitude, and distribution of loading along the sternotomy midline *in vivo* using a porcine model. Changes in forces incurred by death and embalming were also investigated to estimate the applicability of cadavers as chest models for sternal fixation. Two instrumented plating systems were used to measure the magnitude, direction, and distribution of forces across the bisected sternum in four pigs during spontaneous breathing, ventilated breathing, and coughing for four treatments; live, dead, embalmed, and refrigerated. Forces were highest in the lateral direction and highest at the xiphoid. An important finding was that the magnitude of the respiratory forces in all directions was smaller than anticipated from previous estimations, ranging from 0.37 N to 43.8 N. No significant differences in force were found between the four treatments, most likely due to the very small magnitude of the forces and high variability between animals. These results provide a first approximation of *in vivo* sternal forces and indicate that small cyclic fatigue loads should be applied for long periods of time, rather than large quasistatic loads, to best evaluate the next generation of sternal fixation devices.

Table of contents

List of figures

List of tables

1. Introduction

Median sternotomy, used by cardiothoracic surgeons to access the heart during surgery, requires that the bisected sternum be reapproximated post-surgery using a fixation device. Although standard wire fixation is successful in most patients, complications associated with inadequate fixation such as sternal discomfort, mediastinitis, and dehiscence affect approximately 15,000 Americans every year (A.H.A., 2004; Stahle, et al., 1997) and of this population 10-40% risk death (El Oakley and Wright, 1996). Despite recent promising investigations of rigid alternatives to wires to reapproximate the sternum (Centofanti, et al., 2002; Gottlieb, et al., 1994; Ozaki, et al., 1998; Stahle, et al., 1997; Tavilla, et al., 1991), limited mechanical study and uncertainty about the stability that these novel devices provide has diminished their widespread clinical use.

Current testing of the efficacy of sternal closure techniques is typically conducted *in vitro* because it is a rapid, controlled, inexpensive, and less variable alternative to using cadavers and animal models. Mechanical stability afforded by different fixation techniques is usually quantified by quasistatically applying estimated *in vivo* loads to a model system and observing the separation at the midline (Dasika, et al., 2003; Losanoff, et al., 2004; Ozaki, et al., 1998; Trumble, et al., 2002). This separation is assumed to be indicative of micro-motion at the wound site which is thought to be a critical factor during healing (Chakkalakal, et al., 1999; Claes, et al., 2002; Yamaji, et al., 2001). However, the simple loading conditions applied *in vitro* do not appear to adequately represent the complex loading of the sternum *in vivo*; we previously noticed uncharacteristically large separations at the xiphoid region in comparison with clinical observations with near-uniform lateral loading of wire-fixed sternal models (Pai, et al., 2005). An estimation of the *in vivo* forces on the sternal midline based on Wolff's Law indicated that the large xiphoid distractions were an artifact of the simplified loading rather than due to inadequate fixation in this area (Pai, et al., Submitted). Since previous test methods do not necessarily reproduce the loading *in vivo* because these forces have yet to be

characterized, the outcomes of these tests have limited physiological relevance and cannot fully assess the potential clinical shortcomings of novel fixation devices.

In order to improve current *in vitro* test methods, knowledge of the direction, magnitude, and distribution of loading along the sternal midline *in vivo* are necessary. Forces that are placed on the sternum include respiratory forces due to breathing and coughing (Casha, et al., 1999). As it is not practical to measure these forces in humans as it would require invasive surgery, preliminary measurements must be conducted in a model system. Pigs have previously been used as a model of the human chest (Losanoff, et al., 2002; Trumble and Magovern, 2004) and are an acceptable model for a preliminary investigation. However, if the effects of rigor mortis and fixation were quantified and found to be insignificant with respect to living conditions, cadavers would be a more anatomically accurate model for future tests. Thus, the purpose of this initial study is two-fold; (i) to quantify the magnitude, direction, and distribution of relevant *in vivo* respiratory forces on the porcine sternum that should be used for future evaluation of sternal fixation devices and (ii) to investigate the accuracy of using cadavers as a future sternal model system.

2. Background

2.1. The sternum and midline sternotomy

The sternum, or breastbone, is located in the center of the ventral thorax anterior to the vertebral column and connects to the upper seven pairs of ribs. It is a flat bicortical bone with an outer cortical shell and an inner cancellous layer containing bone marrow for producing blood; an important factor post surgery because excessive blood loss and wound exposure increases the risk of complication (White, 2000). The three parts of the sternum are the manubrium which is the densest part of the sternum and articulates with the clavicle, the corpus which is where rib pairs two through seven attach, and the xiphoid which is the soft cartilaginous part of the sternum (Figure 1A).

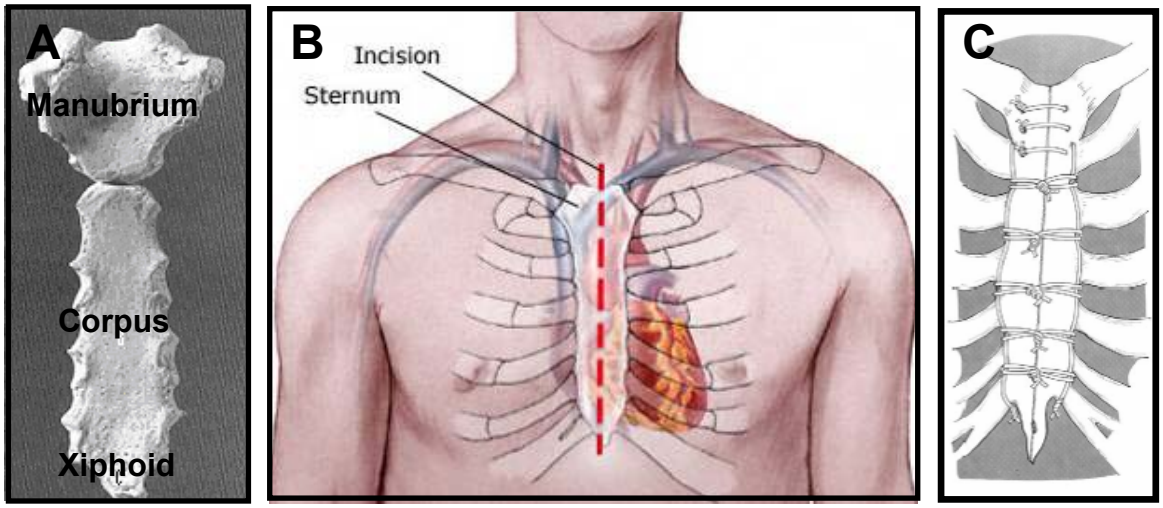


Figure 1: Schematic of sternum with (A) locations of manubrium, corpus and xiphoid process (White, 2000) (B) site of midline incision (Guidant, 2004) and (B) cerclage wire fixation (Molina, et al., 2004).

Median sternotomy is the standard procedure used to access the heart whereby the sternum is bisected to reveal the organs within the chest cavity (Figure 1B). Post surgery the sternum is reapproximated with a sternal fixation device, typically stainless steel cerclage wires (Figure 1C). Stable fixation is critical as sternal complications such as dehiscence (painful fracture separation without infection, Figure 2A), osteomyelitis (superficial wound infection), and mediastinitis (deep infection of the chest cavity, Figure 2B) may arise if the sternum is not immobilized adequately

(Bryan, et al., 1992; Karp, 1996; Loop, et al., 1990; Mayba, 1985; Stoney, et al., 1978). Motion at the wound site has adverse effects on the healing process (Sargent, et al., 1991) and only a fracture separation of a few millimeters is thought to be allowable for bony healing to occur (Chakkalakal, et al., 1999; Claes, et al., 2002; Yamaji, et al., 2001). Patients who are predisposed to poor bone healing such as those with osteoporosis (weak and brittle bones), diabetes (retarded blood circulation and healing), and emphysema (large coughing forces) are at higher risk of complications (El Oakley and Wright, 1996; Stahle, et al., 1997).

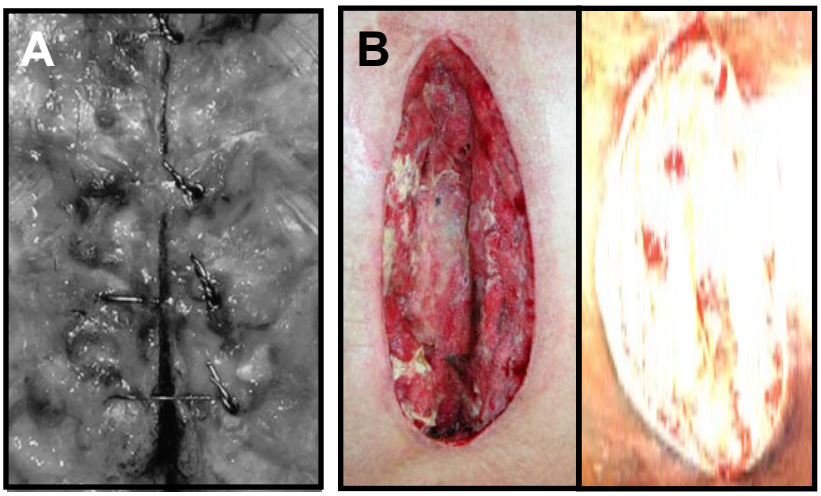


Figure 2: Post-operative complications include (A) dehiscence and pull-through of wires (Ozaki, et al., 1998) and (B) mediastinitis (deep infection).

2.2. Alternate sternal fixation techniques

In an attempt to reduce sternal wound healing complications, some surgeons have turned to alternative more rigid sternal fixation techniques (Figure 3) that are thought to promote faster healing thereby reducing the likelihood of post-operative complications sternum (Centofanti, et al., 2002; Gottlieb, et al., 1994; Ozaki, et al., 1998; Stahle, et al., 1997; Tavilla, et al., 1991). Of these methods, rigid metal plates have been investigated most actively (Gottlieb, et al., 1994; Ozaki, et al., 1998; Sargent, et al., 1991) due to their established use in reapproximating almost every other fractured bone in the body (Baugmart and Perren, 1994; Cooper, et al., 1988; Ouellette, et al., 1994). Despite recent studies reporting the clinical success of rigid metal plates (Hendrickson, et al., 1996; Smoot

and Weiman, 1998; Song, et al., 2004), stainless steel wires remain the standard fixation technique because they are simple to use, effective for most patients, and there are few mechanical analyses to support the use of more novel fixation methods.

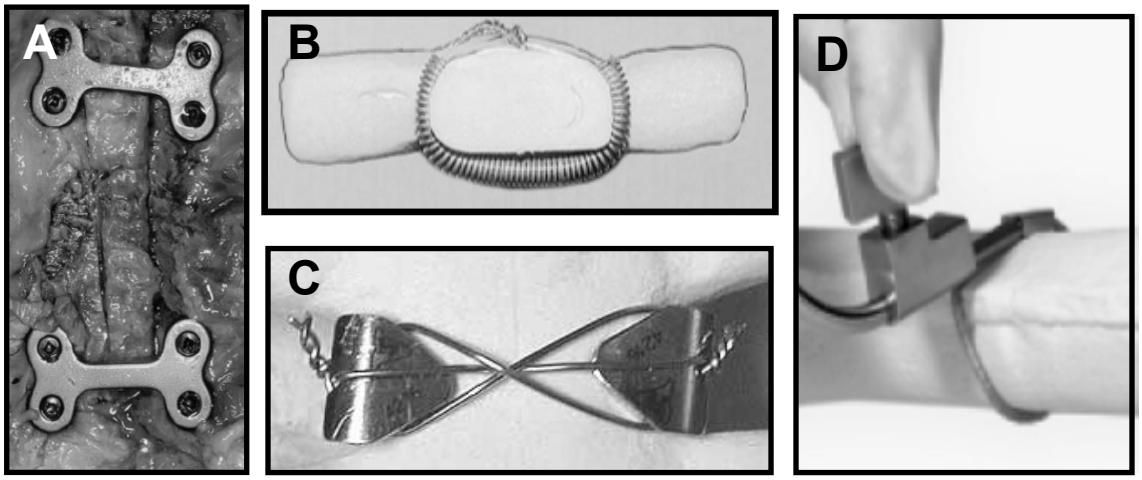


Figure 3: Various alternative fixation techniques to wires including (A) rigid metal plates (Ozaki, et al., 1998), (B) reinforced steel wires (McGregor, et al., 2003), (C) dynamic sternal fixation (Cohen and Griffin, 2002), and (D) Dall-Miles cable system (Eich and Heinz, 2000).

2.3. Test methods for evaluating fixation devices

In an effort to provide surgeons with substantial comparative information about the efficacy of different fixation devices, researchers have conducted studies to test the stability provided by the various fixation techniques (Figure 4). While clinical tests (Figure 4A) offer the most pertinent indicator of the success or failure of a device (Song, et al., 2004), they are limited to devices that have already been developed and approved by required legislation and offer little insight into the underlying mechanics governing their performance. Studies conducted in animals (Figure 4B) provide more investigative flexibility for mechanical study (Sargent, et al., 1991) but are an expensive, variable, and slow method for testing many fixation devices. Moreover, they are limited by anatomical differences from humans in chest wall size and shape to a varying degree depending on the particular animal. Cadavers are an ideal anatomical model (Figure 4C) that have been used previously (McGregor, et al., 1999) however they too are expensive, have considerable biological

variation, and it is unknown to what extent rigor mortis and chemical fixation cause physiological differences (Casha, et al., 1999). More recently numerical simulations such as the finite element method (FEM, Figure 4D) have been employed (Bruhin, et al., 2005) because they provide a rapid and inexpensive means of comparing infinite fixation techniques. However, these models are only as accurate as the geometry, loading, and material property data utilized which are often overly simplified. Finally, i*n vitro* testing systems are the most common way of testing fixation devices because they are controlled, precise, rapid and inexpensive.

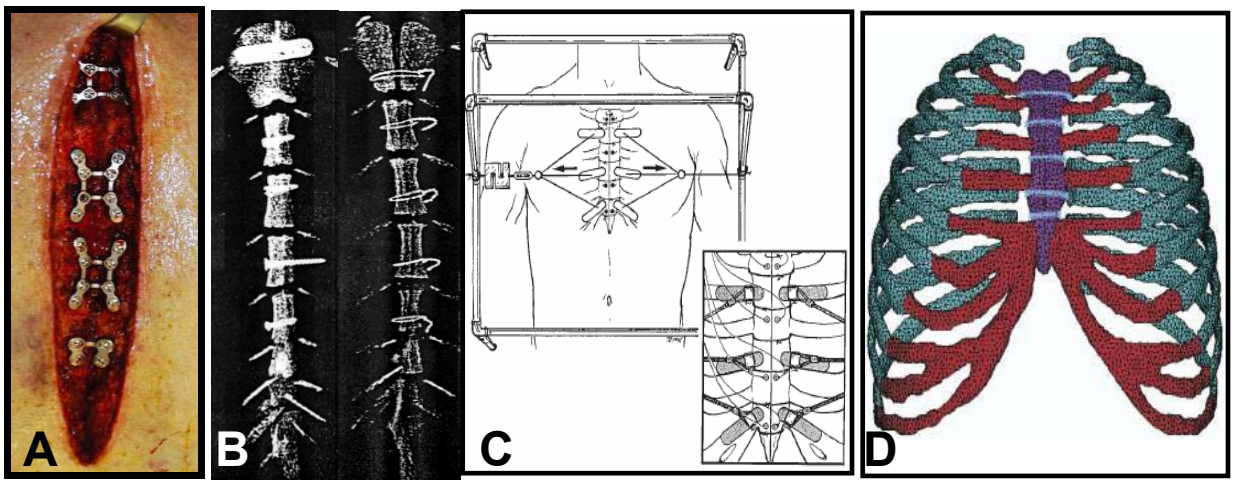


Figure 4: Fixation device evaluation methods include (A) clinical studies (plate fixation (Song, et al., 2004)), (B) animals (x-rays of plate and wire fixation in baboons (Sargent, et al., 1991)), (C) cadavers (wire fixation (McGregor, et al., 1999)), and (D) numerical methods (FEM of wire fixation, (Bruhin, et al., 2005)).

Of the *in vitro* tests conducted to measure sternal stability, two of the main factors used in determining the mechanical stability of a sternal fixation device are the amount of micro-motion the device allows (sternal separation or stiffness) and the strength to failure (pull-out strength) of the device using either cadaveric, porcine, or synthetic (e.g. polyurethane) sterna (Casha, et al., 1999; Cohen and Griffin, 2002; Dasika, et al., 2003; Losanoff, et al., 2004; McGregor, et al., 1999; Ozaki, et al., 1998; Pai, et al., 2005; Trumble, et al., 2002). Despite similarities in principle, there is no standard method of testing sternal fixation devices, and there is considerable variation between loading regimes (Figure 5) ranging from four-point bending tests (Ozaki, et al., 1998), three-

directional catastrophic loading (Cohen and Griffin, 2002), and lateral loading (Pai, et al., 2005; Trumble, et al., 2002). Further, we observed phenomena in our *in vitro* tests (Pai, et al., 2005) that have not been reported *in vivo* including separations on the posterior side of the sterna for plated models and excessively wide separations at the xiphoid for wired models. These discrepancies further highlight the fact that current *in vitro* testing systems may not necessarily represent the complex loading *in vivo*. Until the forces across the sternal midline are characterized, it will be difficult to develop a more standardized loading regime for *in vitro* testing.

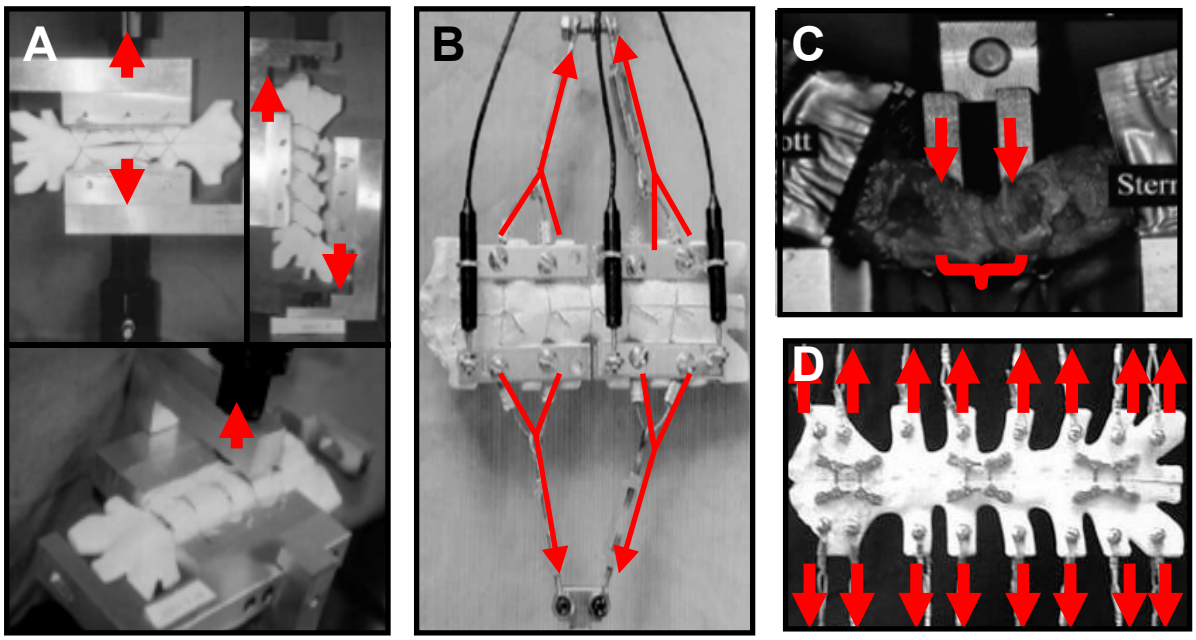
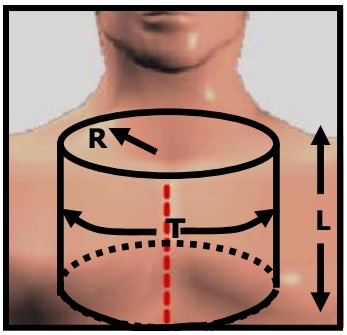


Figure 5: *In vitro* testing systems used to evaluate sternal fixation devices: (A) three directional loading in the lateral, longitudinal shear and transverse shear directions (Cohen and Griffin, 2002), (B) lateral loading (Trumble, et al., 2002), (C) four-point bending compression (Ozaki, et al., 1998), and (D) uniform lateral loading (Pai, et al., 2005).

2.4. Estimations of sternal midline loading

Researchers have suggested that the largest loads placed on the sternum are induced by coughing, sneezing, or impact (Casha, et al., 1999). Estimations of the magnitude of force exerted on an adult sternum due to intrathoracic pressure have been calculated using the Law of Laplace (Figure 6). According to this law, breathing forces range from 160 N to 400 N (Dasika, et al., 2003; Trumble, et al., 2002) and coughing forces range from 550 N for a normal cough to 1650 N for a maximal cough.



Application of Law of Laplace

Tension (T) across sternal midline equals product of chest radius (R), chest length (L), and pressure in chest (P):

$$T = RLP$$
$$\approx (0.15\ m)(0.25\ m)(40\ kPa)$$
$$\approx 1500\ N$$

Figure 6: Estimation of force magnitude across the sternal midline *in vivo* (Casha, et al., 1999).

2.5. Chest wall mechanics

The respiratory forces on the sternum are complex because they are a product of several factors including the rotation of the ribs at the spine and sternum as well as the contraction and relaxation of several muscles of respiration. During inspiration, the ribcage shifts cranially and increases its volume to allow the lungs to expand as they fill with air (Figure 7).

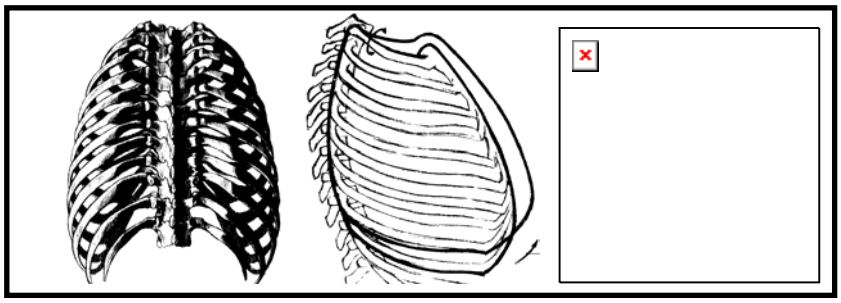


Figure 7: Skeletal anatomy of thorax showing rib attachments to sternum and spine and changes in anatomy during inhalation (Feher, 1996).

This increase in volume occurs as the diameter of the ribcage increases in the lateral and dorsal-ventral directions (Figure 8) by rotating the upper and lower ribs about the spine. It is likely that the forces that act on the ribcage and sternum might act along these directions as well.

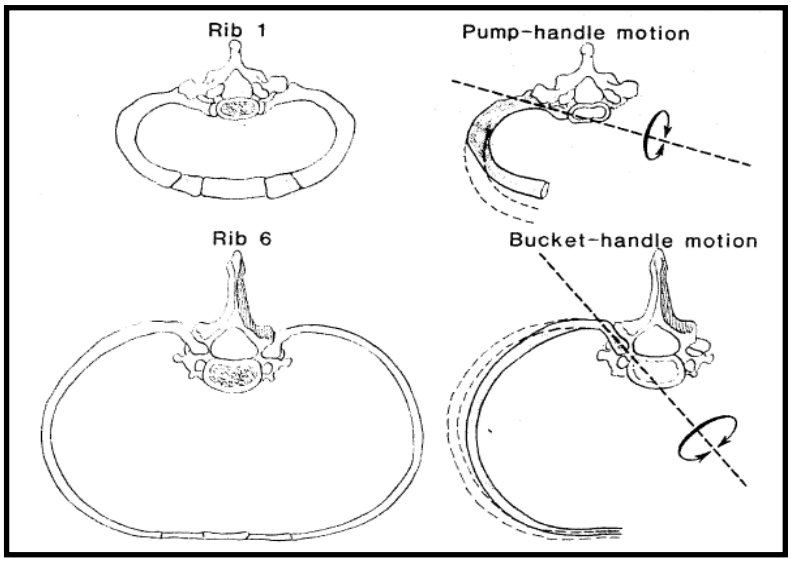


Figure 8: Anatomy of ribcage showing rotation of rib-neck axis during inspiration increases the dorsal-ventral diameter of the upper rib cage (top, pump-handle motion) and the lateral diameter of the lower rib cage (bottom, bucket-handle motion) (De Troyer and Estenne, 1988).

The diaphragm is considered to be the primary muscle of respiration (De Troyer, 1989). During expiration, the abdominal muscles push the visceral mass (organs within the abdomen) up against the diaphragm (Figure 9) in what is known as the zone of apposition. The diaphragm transfers the pressure from the zone of apposition to the thorax, thereby applying an external pressure on the lungs and forcing them to exhale (De Troyer and Estenne, 1988). Thus the abdominal muscles play an important role in respiration (Mier, et al., 1985).

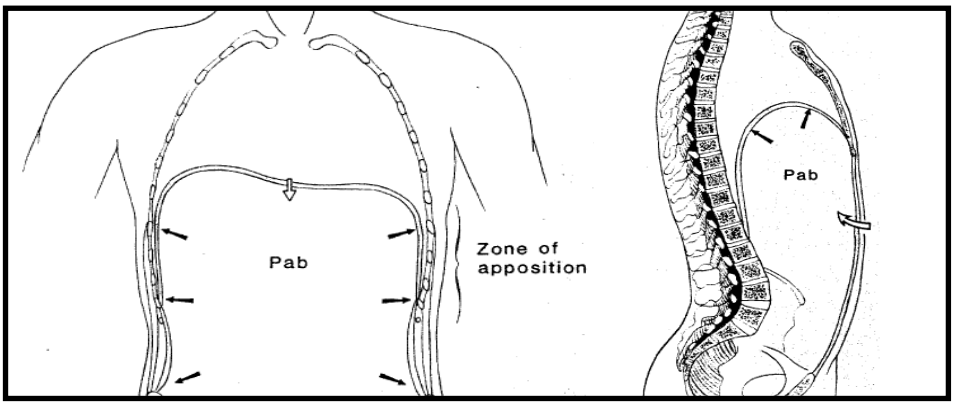


Figure 9: Abdomen and thorax showing how the zone of apposition (abdomen) can push the diaphragm towards the chest to create an expiratory pressure (De Troyer and Estenne, 1988).

The intercostal muscles and scalenes are also important muscles of respiration that are necessary for both inspiration and expiration (Figure 10). These muscles are interwoven along the ribs and have insertion points at both the spine and sternum and so are likely to affect the forces along the sternal midline.

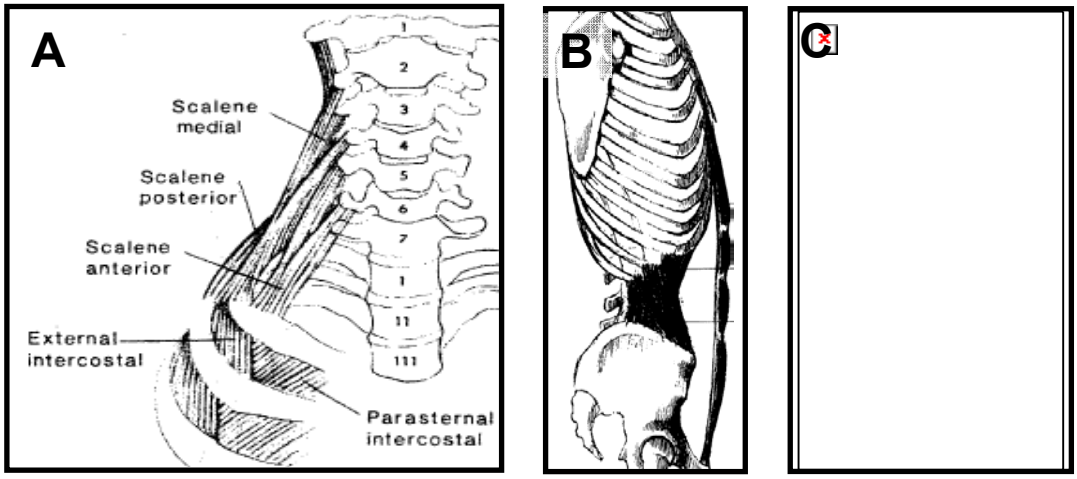


Figure 10: Musculature surrounding sternum that is necessary for spontaneous breathing and potentially contributes to the forces along the sternal midline (De Troyer and Estenne, 1988; Feher, 1996).

In summary, the interactions between the thoracic skeletal system and musculature during respiration are complex and are still under investigation (De Troyer, et al., 2005). Previous models of these interactions (Andriacchi, et al., 1974; Loring and Woodbridge, 1991; Roberts and Chen, 1970; Sundaram and Feng, 1977) and current estimations of the loading on the sternum have simplified these interactions considerably because it is difficult to predict how all of these different muscles combined with the complex kinematics of the bones, will translate into forces on the midline of a bisected sternum. As a result of these difficulties, it would appear that the simplest way to approximate sternal midline loading would be to physically measure these forces in a representative living system.

3. Specific Aims and Rationale of Project Approach

The goal of this project was to characterize the physiological forces that occur on the sternum midline *in vivo* following median sternotomy in a porcine model. Results from this study will provide inputs for application in finite element and *in vitro* sternotomy models used to evaluate sternal fixation devices. Such improvements to these models will aid in determining the optimal usage and development of sternal fixation devices. Previous device evaluation methods have been limited by potentially inaccurate loading conditions, since these forces are not known.

Rationale for experimental model

Although cadavers are anatomically the most appropriate model, it is unclear whether they are a good approximation of the physiological force conditions in a living model. Clinicians speculate that the effects of rigor mortis and chemical fixation greatly alter the forces on the sternum due to changes in chest wall compliance as fixatives such as formaldehyde and formalin have been reported to affect muscle and bone material properties (Wilke, et al., 1996). Conversely, living animal models are limited by anatomical differences but are more physiologically appropriate. Primates were too expensive to use in this study. Dogs have typically been used in respiratory research (De Troyer, 1989; De Troyer and Decramer, 1985; De Troyer, et al., 2005; De Troyer and Wilson, 1993) however, recent comparisons show that pigs are a more appropriate large animal model of the thorax (Figure 11A) due to chest wall size and shape (Cook, et al., 1996; Trumble and Magovern, 2004). While sheep are another popular large animal model, their chest walls are less similarly shaped to humans than pigs' are (Popesko, 1977). Although pigs (and other quadrapeds) are limited because of considerable differences in gross anatomy compared to humans (Figure 11B), they have previously been used as sternal and thoracic models (Becker, et al., 1972; Losanoff, et al., 2002) and, due to their aforementioned chest wall size and shape, we found they are an acceptable model for a first approximation of sternal midline loading.

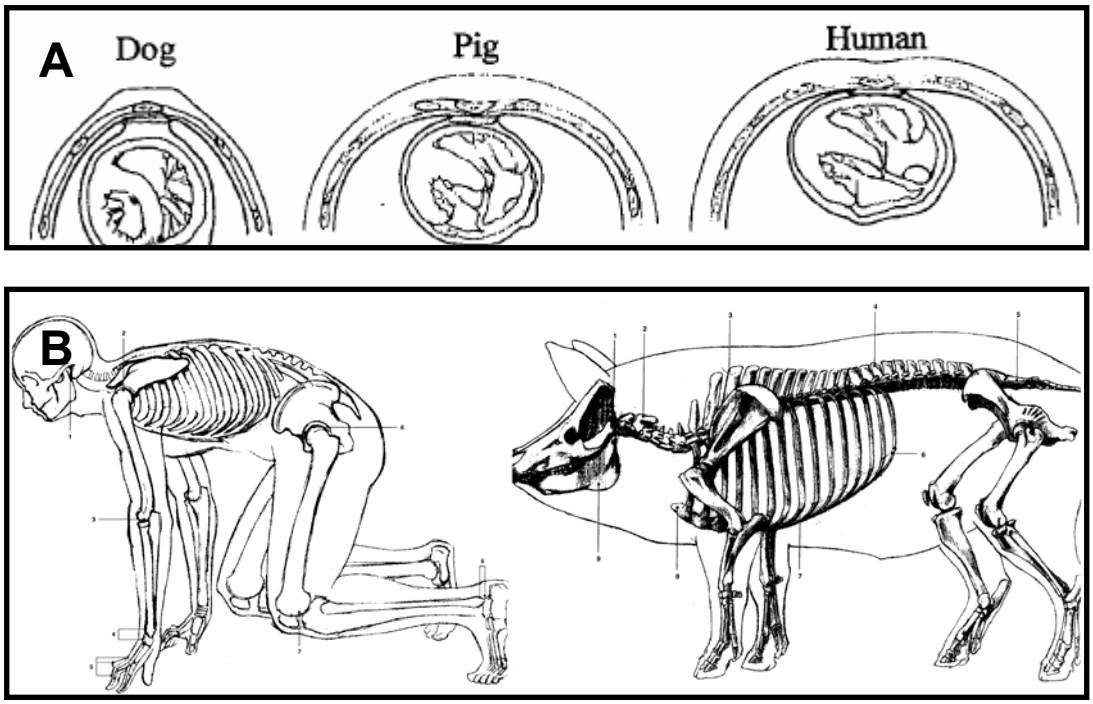


Figure 11: Differences in animal anatomies: (A) comparison of dog, pig and human thorax (Trumble and Magovern, 2004), (B) comparison of human and pig gross skeletal anatomy showing differences between bipeds and quadrapeds (Feher, 1996).

Specific aim 1: **Quantify the distribution, magnitude and direction of *in vivo* sternal forces during breathing and coughing.**

Rationale: We measured respiratory forces because these forces continually act on the sternum and should be mimicked in a sternal closure testing system. Coughing forces were important to measure because they are believed to exert the maximum forces on the sternum due to the high intrathoracic pressures. We measured the force distribution along the sternum because it is not known whether the manubrium or xiphoid should be loaded more heavily in sternal closure testing systems (Figure 12A). Finally, we measured the direction of the forces (Figure 12B) because it seemed likely that each direction would affect the separation and hence stability at the sternal midline.

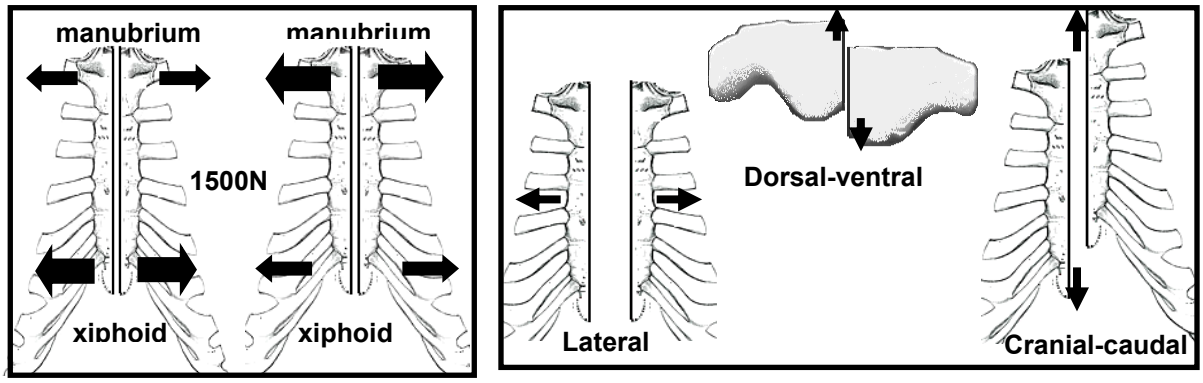


Figure 12: Schematic showing potential (A) force distribution, magnitude, (B) and direction (Gray, 1918).

Based on estimations made by previous researchers, we expected the forces across the sternum to be between 200 to 1500 N so we purchased a series of 222 N (50 lb) tension-compression force transducers. However, preliminary measurements with these transducers in dead ventilated pigs indicated that the forces may be much lower in range (0-25 N) hence we used lower range force transducers (22.2 N (5lb)) for the actual study. We placed the transducers in three orthogonal directions; one in each shear direction (dorsal-ventral and cranial-caudal) and two in the lateral direction on either end of the sternum to characterize both the direction and distribution of forces.

Since the sternum is not a level surface, we anticipated that it would be difficult to align the transducers in the chosen directions. Thus, we placed the transducers on metal plates before attaching them to each sternal half and we used bone cement as a filler to create a more level platform for better transducer alignment. Although we originally considered using force transducers capable of measuring in three directions to reduce the bulkiness of our set-up, we were limited by budget constraints and our inability to find small enough transducers. We also considered building our own transducers using triple-rosette strain gauges however due to time constraints we recognized that we would not be able to match the high sensitivity of the force transducers that are commercially available (0.15% FS).

Specific aim 2: **Determine the effect of spontaneous breathing vs. ventilated breathing on sternal midline loading.**

Rationale: Since sternotomy patients are put on ventilators for substantial periods of time before being expected to breathe normally, future *in vitro* device evaluation methods will need to recreate both forces. Hence, both these forces were important to measure. Additionally, we reasoned that a difference in response would help estimate the plausibility/ limitations of using cadavers in future models since they need to be ventilated to simulate breathing.

Specific aim 3: **Investigate the difference between live vs. cadaveric sternal midline loading.**

Rationale: Since cadavers have more anatomically similar chest walls to living human than any large animal model, the use of cadavers in lieu of live animals in future biomechanical studies would increase the anatomical relevance of the studies and minimize animal use and cost. However, the physiological changes from the living condition to cadaveric state have not been characterized with respect to sternal forces. By comparing the forces under four treatments in an animal model (living, dead, embalmed, and refrigerated), we hoped to translate the results to humans to evaluate the differences between the living condition, fresh cadavers and fixed cadavers.

4. Materials and Methods

4.1. Experimental protocol

The experiments were conducted in compliance with the University of Massachusetts Medical School Institutional Animal Care and Use Committee on four female Yorkshire pigs weighing between 49 and 68 kg (109 and 150 lb). All animals were prepped by fasting for 24 hours prior to surgery and were anaesthetized with 1 ml/20 kg of a solution of Telazol (2 mg/kg), Ketamine (1 mg/kg), and Xylazine (1 mg/kg), placed on a heating pad in the supine position, intubated, and kept hydrated by means of a Novalon ear vein catheter supplying saline at 300 ml/hr. Inhaled 1.5%-2.5% Isofluorane was used for continuous anesthesia. Standard midline sternotomy techniques (Julian, et al., 1957) were used to separate the sternum while minimizing disturbance of surrounding musculature (Figure 13A). Two specially designed plating systems used to measure the sternal forces for several treatments using the same three animals per group were attached sequentially to the sterna by means of bone screws and bone cement (Depuy Orthopaedics, Warsaw, IN).

4.2. Force measurement system

The first plating system (Figure 13B) was designed to monitor the direction of forces along the sternum using four 50 lb (222 N) force transducers (Model 31, Honeywell-Sensotec, Columbus, OH) placed longitudinally, laterally (m-lateral at the manubrium and x-lateral at the xiphoid), and vertically. The second plating system (Figure 13C) was used to monitor the distribution of the lateral forces using more sensitive 5 lb (22.2 N) force transducers (Model 31, Honeywell-Sensotec, Columbus, OH). Additionally, a 50 psi (345 kPa) surface pressure transducer (EPL-B02-10P-/X, Entran Devices, Inc., Fairfield, NJ) was placed on the posterior side of the sternum to monitor the local intrathoracic pressure in one animal and a 50 psi (345 kPa) pressure transducer (AB-HP, Data Instruments, Acton, MA) was placed in the trachea to monitor lung pressure in another animal.

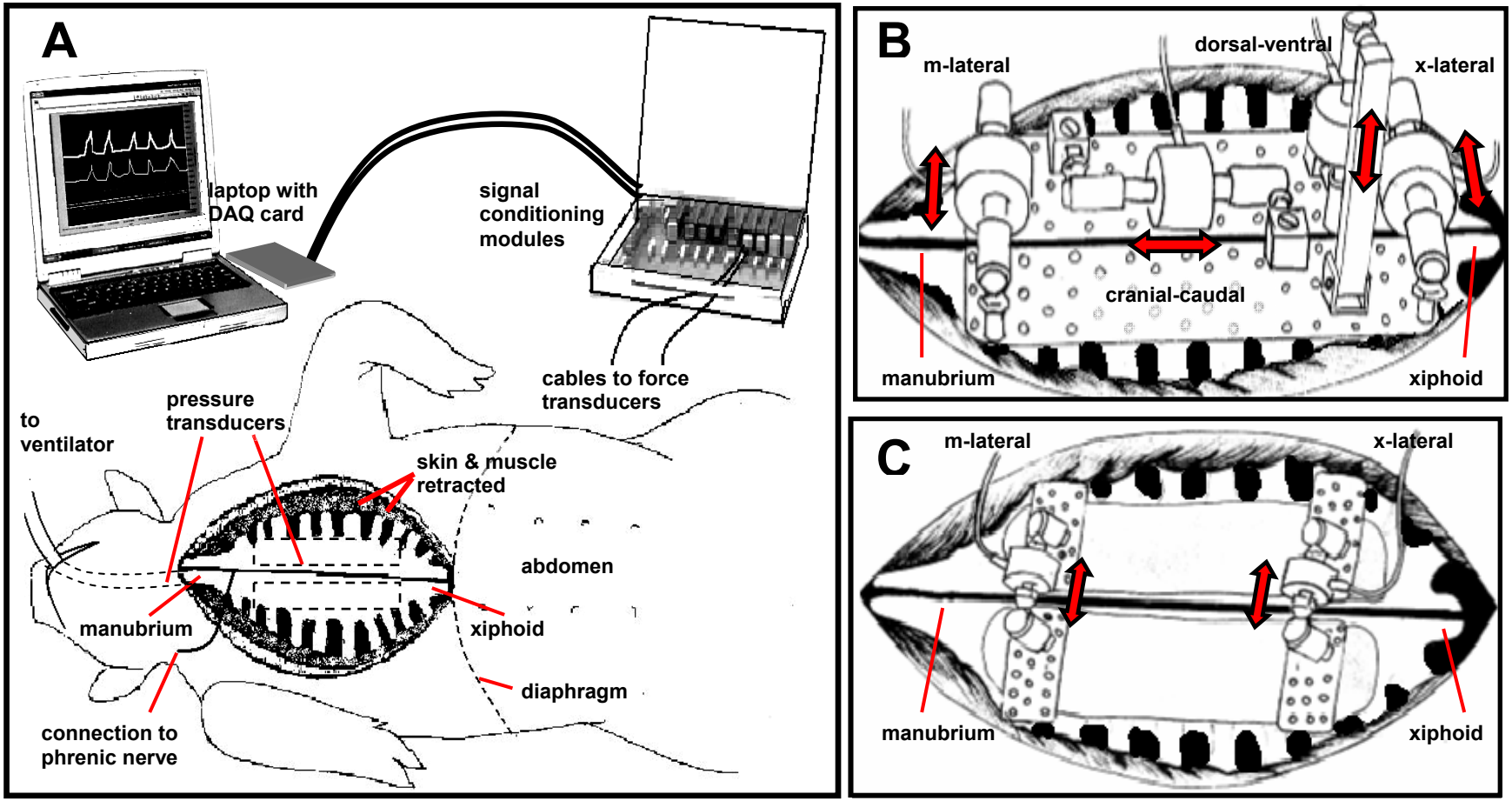


Figure 13: Experimental set-up showing (A) supine pig with sternum exposed and instrumentation used to measure the forces: a valsalva force was applied at the abdomen near the diaphragm and a phrenic cough was stimulated by applying a voltage to the phrenic nerve. An outline of the directional plating system is shown on the sternum. (B) Close-up of plating system used to measure forces in three orthogonal directions as indicated by the arrows; 222 N (50 lb) force transducers are shown from left to right in the lateral direction at the manubrium, cranial-caudal and dorsal-ventral directions at the midsternum, and lateral direction at the xiphoid. (C) Distributional plating system with 5 lb (22.2 N) force transducers placed laterally at the manubrium and xiphoid. Note that ball-joints were attached to the force transducers from both plating systems to allow free movement in the directions not being measured.

Signals from all force and pressure transducers were filtered (1.6 kHz lowpass filter) and amplified externally (SC-2345 and SCC-SG24, National Instruments, Austin, TX) before acquisition using a 12-bit multifunction DAQ board (NI PCI-6221 M-Series, National Instruments, Austin, TX) at frequency of 100Hz during breathing and ventilation and 500Hz during coughing. Data were recorded using LabVIEW (National Instruments, Austin, TX) and static forces (expiratory baseline) were separated from dynamic forces (inspiratory peak, Figure 14) using MATLAB 7.0 (The MathWorks Inc., Natick, MA).

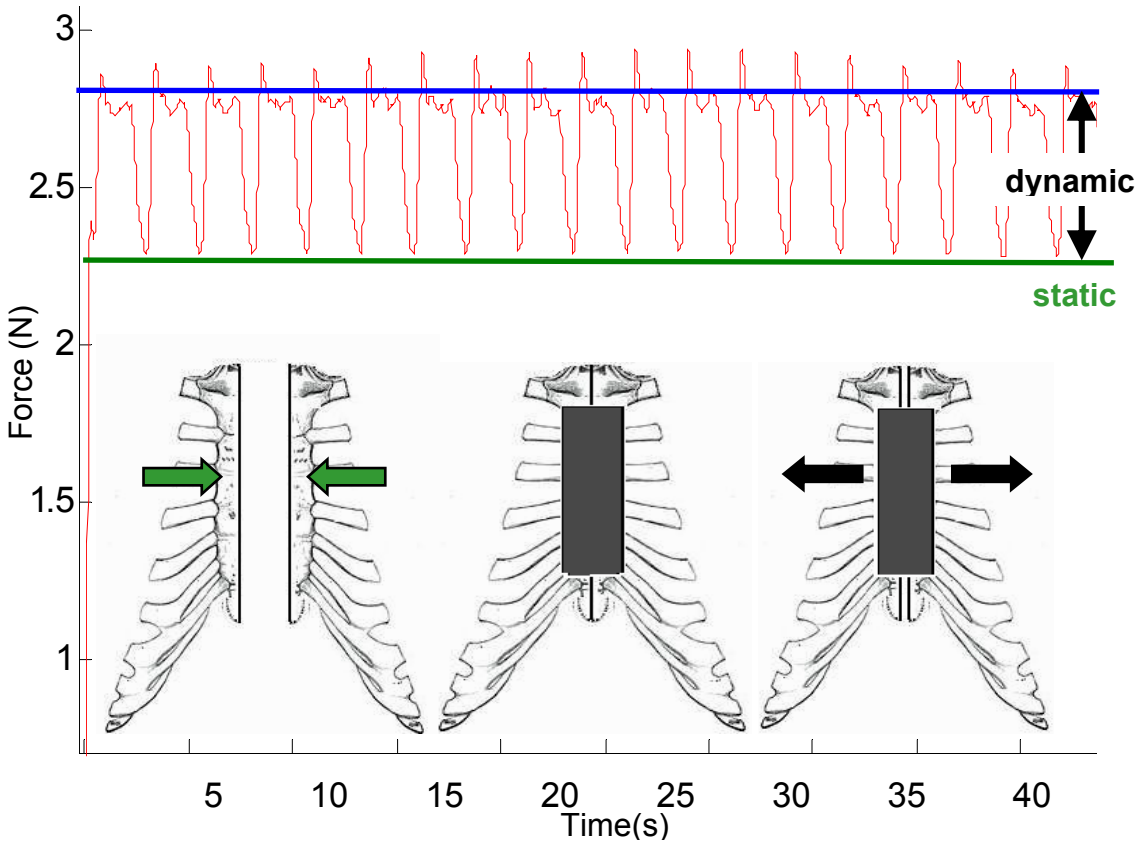


Figure 14: Schematics showing sample respiratory waveform in red. Static forces are equivalent to the signal base values (shown in green) and represent the force required to bring both sternal halves together without effects of breathing. Dynamic forces are equivalent to the amplitude of the signal (shown in black) and represent the additional force required to hold the sternal halves together due to respiration/coughing.

4.3. Treatments

Forces were measured under the treatments and measurement groups described below in Table 1.

Table 1: Summary of different measurement groups within each treatment

TREATMENT	***MEASUREMENT GROUPS ****			
	1	**2**	**3**	**4**
Live-Spontaneous (L-S)	unretracted	retracted	valsalva cough	phrenic cough
Live-Ventilated (L-V)	20 cmH_2O	30 cmH_2O	cough 20 cmH_2O	
Dead-Ventilated (D-V)	20 cmH_2O	30 cmH_2O	cough 20 cmH_2O	
Embalmed-Ventilated (E-V)	20 cmH_2O	30 cmH_2O	cough 20 cmH_2O	40 cmH_2O
Refrigerated-Ventilated (R-V)	20 cmH_2O	30 cmH_2O	cough 20 cmH_2O	40 cmH_2O

** ventilation pressure values shown with units in cmH_20 where $1cmH_20$ = 98.0665 pascal*

The first treatment group we characterized was for animals breathing without the assistance of a ventilator ("Live-Spontaneous"). The effect of retracting the sternal halves apart was investigated in the first animal by measuring the forces before and after retractors were used and removed; retracted sternal halves were separated approximately 4-5 cm and fully retracted sternal halves were separated by about 10 cm. All subsequent measurements were considered retracted. The effect of coughing was investigated using two methods; first by electrically stimulating the phrenic nerves in the neck and second, by manually applying pressures to the abdomen to simulate a valsalva cough. The effect of spontaneous versus ventilated breathing was investigated by placing the animals on a ventilator for the "Live-Ventilated" groups. Several pressures were used to investigate the effect of intrathoracic pressure on sternal force (pressures ranged from 20 to 30 cmH_2O; SpO_2 was monitored as an indicator of appropriate ventilation). The effects of death were investigated by euthanizing the animals, storing them for approximately four hours to ensure similar effects of rigor mortis to a fresh cadaver, and then measuring the forces ("Dead-Ventilated" group). Similarly, the effects of chemical fixation were investigated by embalming the animals using embalming fluid typically used to preserve research-grade cadavers (37% formaldehyde, Cornell Wetting Solution, Hydrol Chemical Co., Yeadon, PA)

and repeating the measurements ("Embalmed-Ventilated" group). Embalming was performed by first flushing 20-30 ml of 10,000 units of heparin/ml via catheterization of the common carotid artery to minimize blood clotting and allow more complete perfusion. Permaflow (Dodge Chemical Co., Cambridge, MA) was then pumped through the carotid followed by the embalming fluid at a flow rate of 3ml/minute by means of a peristaltic pump until the animals were firm to touch (~4 L of fluid). Finally, the animals were refrigerated for 24 hours at 4ºC before taking the final measurements for the "Refrigerated-Ventilated" groups.

4.4. Estimation of the distribution of loading along the sternal midline

To estimate the force distribution in our previous *in vitro* device evaluation system(Pai, et al., Submitted), we idealized the polyurethane model as a homogeneous, linearly elastic solid loaded at multiple locations representing the rib struts. By St. Venant's principle, the concentrated loads at the screws were assumed to be distributed evenly at the rib strut/sternum junction and equal to the tether force divided by the particular strut area. The Flamant/Boussinesq solution (Malvern, 1969)

$$\sigma_{xi}(x,y) = \pi\left[\int_{c_i}^{d_i} 2q_i \frac{x^2}{[x^2+(y-s)^2]^2}\,ds\right]$$

was used to calculate the stress, σ, produced by each rib perpendicular to the midline of the sternum (x-direction) along its length (y-direction), where rib 'i' extends from location c_i to d_i along the y-direction, and s is an integration variable in the y-direction. The stresses due to the loads at each of the rib struts, q_i, were summed to determine the total stress at each location (x, y), and the load distribution (force/unit length) was calculated by multiplying the stress along the midline (x = 1.2 cm) by the thickness and normalizing to unit length. The simulation was implemented in MathCAD (Mathsoft Engineering and Education Inc., Cambridge, MA).

For a rough comparison with our loading distribution, we estimated the force distribution at the midline of human sterna by measuring the cortical bone thickness along the midline of three cadaver sterna and assuming that the cortical shell bears the majority of the load and that bone forms in proportion to loading (i.e., Wolff's law). The sterna were bisected using standard surgical technique, images were taken using a 6.3 megapixel digital camera (Canon Digital EOS, Japan) and the bone density was quantified using image analysis software (Scion Image, Scion Corporation, Fredrick, MD).

4.5. Statistical analysis

The average respiratory forces (dynamic forces caused by inspiration) for both plating systems were compared for each of the treatments using an analysis of variance (ANOVA) with blocking to isolate animal-to-animal variability (SigmaStat, Systat Software, Inc., Point Richmond, CA). A p-value of 0.05 indicated significant differences between groups which were further analyzed by *post hoc* analysis using the Tukey HSD.

5. Results

In general, the measured forces were small relative to previous estimations and all forces were less than 45 N (~10 lbs). Static forces were larger than dynamic forces in all directions however they remained under 21 N irrespective of treatment. Dynamic forces ranged from 0.37 N (dorsal-ventral, L-S unretracted) to 5.33 N (x-lateral, L-S retracted) except for phrenic coughing when forces as high as 43.8 N (m-lateral) were recorded. The force distribution for each treatment (live, dead, embalmed, and refrigerated) did not vary significantly. Despite noticeable differences in mean force levels between measurement groups, the only statistical differences found were between force directions.

5.1. Static forces are small and consistent

The average static forces for all treatment groups are shown in Table 2 and Figure 15. The forces in the lateral direction, particularly at the manubrium, were 4.5 times higher than the forces in the other directions. However, there were no statistical differences between directions.

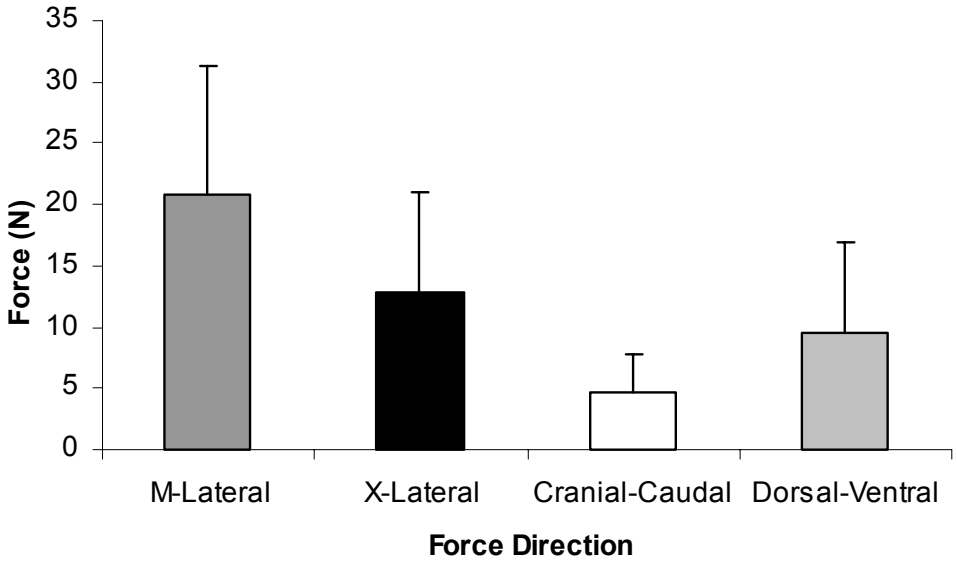


Figure 15: Average static forces for all treatments in each direction.

Table 2: Average static (expiratory baseline) forces for all treatments for each direction (n=3)

DIRECTION	*MEAN ± SD (N)*		
M-lateral	20.85	±	10.53
X-lateral	12.79	±	8.21
Cranial-caudal	4.63	±	3.21
Dorsal-ventral	9.58	±	7.42

5.2. Extent of sternal retraction has limited effect

Data from the first animal (Table 3, Figure 16) indicated that increasing the extent of retraction from unretracted to retracted increased the dynamic forces by over 50% in each direction however, widely retracting the sternum did not further increase the forces. As a result, the subsequent animals' sterna were moderately retracted. Retraction increased the forces by approximately 57% to 160% depending upon direction for all animals.

Table 3: Case study investigating the effect of retraction (n=1)

Retraction level	***INSPIRATORY PEAK (N)***			
(live spontaneous breathing)	***M-lateral***	***X-lateral***	***Cranial-caudal***	***Dorsal-ventral***
Unretracted	0.69	2.42	0.80	0.40
Retracted (4-5 cm)	1.05	5.28	2.42	1.50
Fully retracted (~10 cm)	1.03	5.25	2.31	1.79

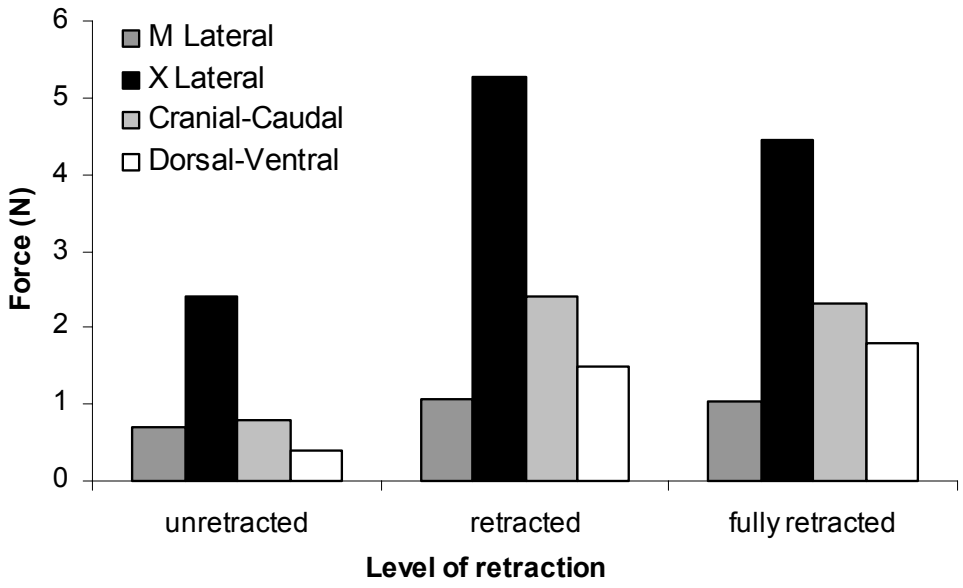


Figure 16: Effect of level of retraction on dynamic forces in all directions in one animal.

5.3. Effect of treatment on force direction and magnitude

The forces in the lateral direction were predominant for all treatments. These lateral forces were highest at the manubrium during the phrenic cough whereas they were highest at the xiphoid for all other treatments with statistical differences in forces for several cases (Table 4).

Table 4: Average dynamic forces for each direction and treatment (n=3)

	MEAN PEAK ± SD (N)			
TREATMENT	***M-lateral***	***X-lateral***	***Cranial-caudal***	***Dorsal-ventral***
L-S unretracted	0.58 ± 0.16	3.40 ± 1.93	1.02 ± 0.41	*0.37 ± 0.12
L-S retracted	*1.42 ± 0.98	5.33 ± 1.81	1.95 ± 0.41	*0.96 ± 0.55
L- phrenic cough	43.8 ± 71.3	35.8 ± 49.2	9.03 ± 9.59	10.0 ± 15.3
L-V 20 cmH_2O	*0.83 ± 0.15	4.15 ± 1.80	2.64 ± 1.21	*0.87 ± 0.44
L- valsalva cough	2.08 ± 1.14	2.69 ± 0.02	3.44 ± 0.72	2.76 ± 2.00

*Note: Significant differences between the x-lateral direction and other directions are marked by *(i.e. across columns within each row)*

Spontaneous breathing did not have significant effects on force in any direction compared to ventilated breathing however the spontaneous breathing forces were slightly larger in the lateral direction (Figure 17).

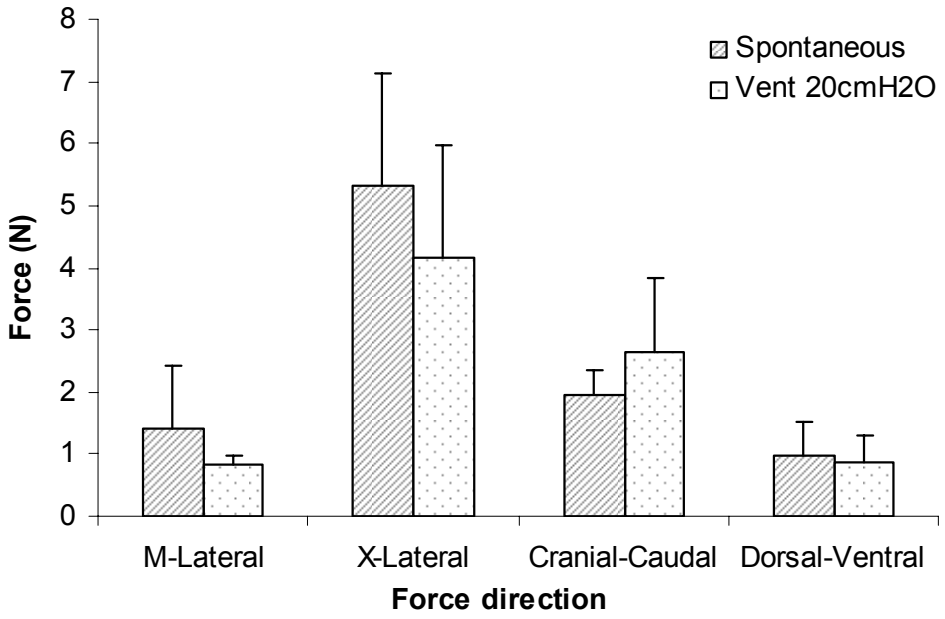


Figure 17: Effect of spontaneous versus ventilated breathing in all directions.

Forces in all directions during the phrenic cough were 1.6 to 20-fold higher than those induced by the valsalva cough. The differences were especially evident in the lateral direction (Figure 18).

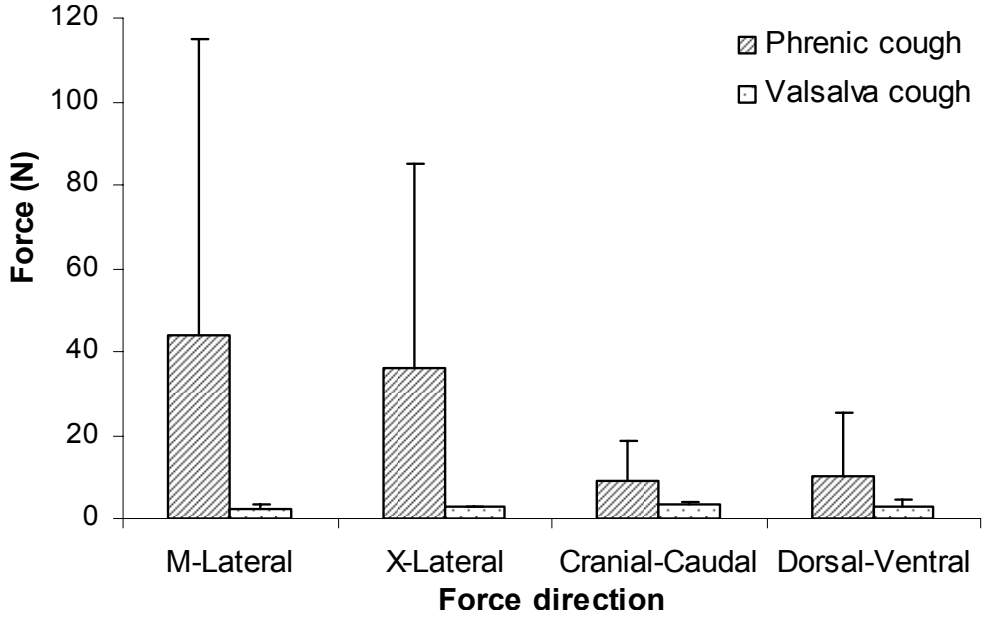


Figure 18: Effect of phrenic cough versus valsalva cough on forces in all directions.

Higher forces were generally observed for the live treatments as compared to the dead, embalmed, and refrigerated treatments (Table 5), although not significantly.

Table 5: Dynamic force distribution as a function of treatment

	MEAN PEAK ± SD (N)					
TREATMENT	**M-Lateral**			**X-Lateral**		
L-V, 20cmH₂O	0.61*	±	0.76	1.32*	±	1.40
L-V, 30 cmH₂O	0.54*	±	0.06	1.35*	±	0.58
L-V, cough 20 cmH₂O	3.99*	±	3.20	3.63*	±	0.31
D-V, 20 cmH₂O	0.15	±	0.06	0.21*	±	0.15
D-V, 30 cmH₂O	0.35	±	0.28	0.30*	±	0.27
D-V, cough 20 cmH₂O	0.79	±	0.84	1.07*	±	0.09
E-V, 20 cmH₂O	0.30	±	0.00	0.24*	±	0.27
E-V, 30 cmH₂O	0.53	±	0.37	0.48*	±	0.42
E-V, cough 20 cmH₂O	1.56*	±	1.40	2.14**	±	-
E-V, 40 cmH₂O	0.58*	±	0.02	0.07**	±	-
R-V, 20 cmH₂O	0.57	±	0.33	0.56	±	0.45
R-V, 30 cmH₂O	0.99	±	1.04	0.94	±	0.76
R-V, cough 20 cmH₂O	2.17	±	2.42	1.35	±	1.32
R-V, 40 cmH₂O	1.03	±	0.48	1.38	±	1.14
R-V, 60 cmH₂O	1.54*	±	2.10	1.60*	±	1.95

*Note: n=3 unless marked by * which indicates n=2 due to animal loss and dash(-) indicates n=1 due to additional loss of data from equipment malfunction*

Within each of the treatment groups, coughing caused the highest forces (Figure 19). There were no significant differences observed between treatments at a constant ventilation pressure of 20 cmH_2O (Figure 20) however, small increases in force were observed with increasing ventilation pressure, most noticeably for the refrigerated treatment (Figure 21).

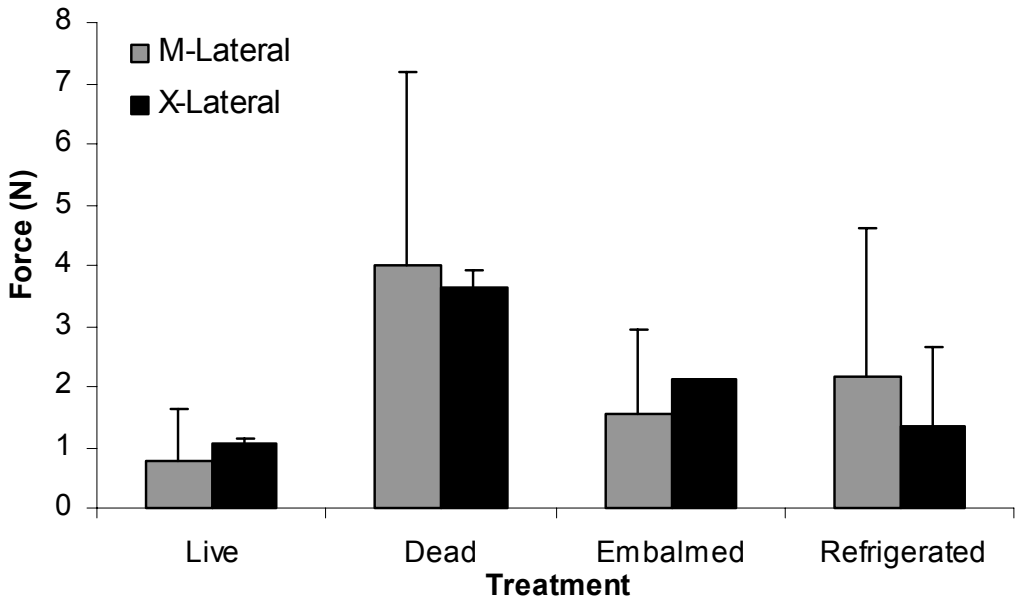


Figure 19: Effect of valsalva coughing on lateral forces for different treatments.

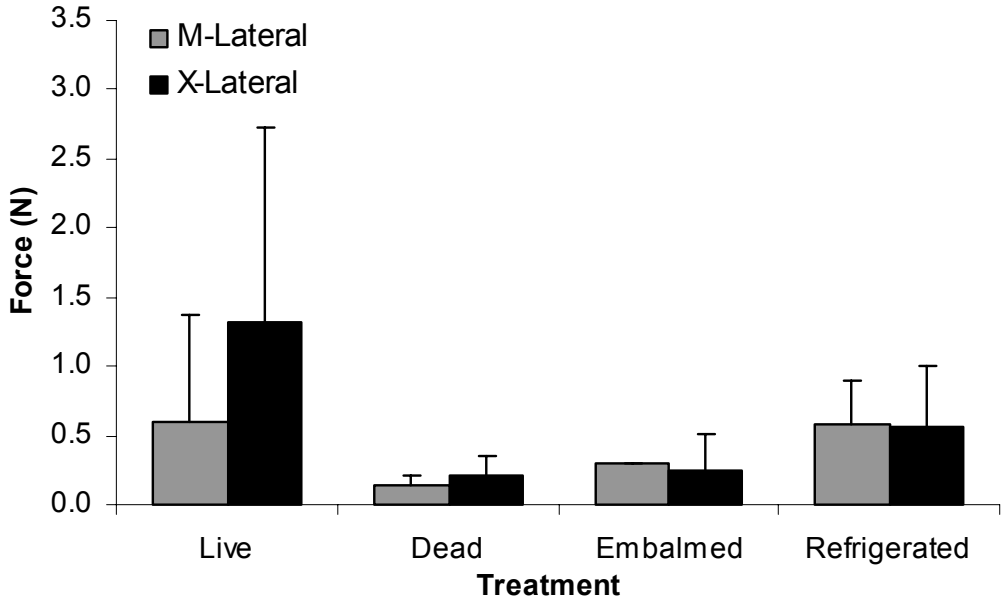


Figure 20: Effect of ventilation at 20cm H_2O on lateral forces for different treatments.

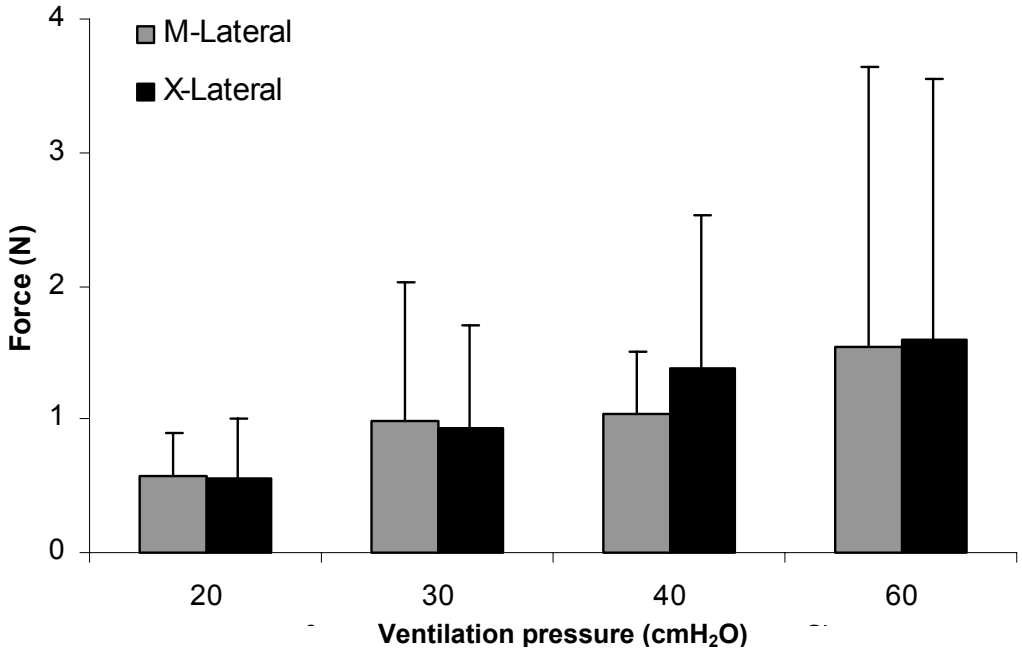


Figure 21: Effect of increased pressure on lateral forces for the refrigerated treatment.

5.4. Intrathoracic pressure varies with treatment

The static pressures on the sternal surface ranged from 2.18 to 5.11 kPa (Table 6). In comparison, the dynamic pressures on the posterior side of the sternum were smaller for all treatments. The dynamic coughing pressures increased by 150% compared to ventilation. Dynamic lung pressures were typically an order of magnitude higher than the corresponding pressures on the sternal surface and showed consistent increases during coughing. Static lung pressures also increased during coughing to a maximum of 38.5 kPa but remained low for all other treatments except for "Live-Ventilated."

Table 6: Case study investigating effect of thoracic pressure (n=1)

Treatment	*STERNAL SURFACE (kPa)*		*LUNG (kPa)*	
	Static	***Dynamic***	***Static***	***Dynamic***
L-V 20 cmH2O	2.30	0.32	31.1	7.51
L-phrenic cough	2.18	0.80	35.0	8.67
D-V, 20 cmH2O	-	-	0.39	1.11
D-V 30 cmH2O	5.11	0.24	0.55	1.60
D-V, cough 20 cmH2O	-	-	38.5	2.11
E-V, 20 cmH2O	-	-	0.52	1.21
E-V, 30 cmH2O	-	-	0.60	1.65
E-V, cough 20 cmH2O	-	-	40.3	4.57

5.5. Sternal density predicts manubrium subject to larger loads than xiphoid

Our analysis of the theoretical force distribution along the sternal midline indicates that the manubrium would be expected to experience larger loads than the xiphoid *in vivo* since it is made of denser and thicker bone (Figure 22, solid line). In comparison with this predicted *in vivo* loading, our previous *in vitro* loading regime using eight points of attachment was relatively consistent in all locations except for the xiphoid which was loaded substantially higher (Figure 22, dotted line).

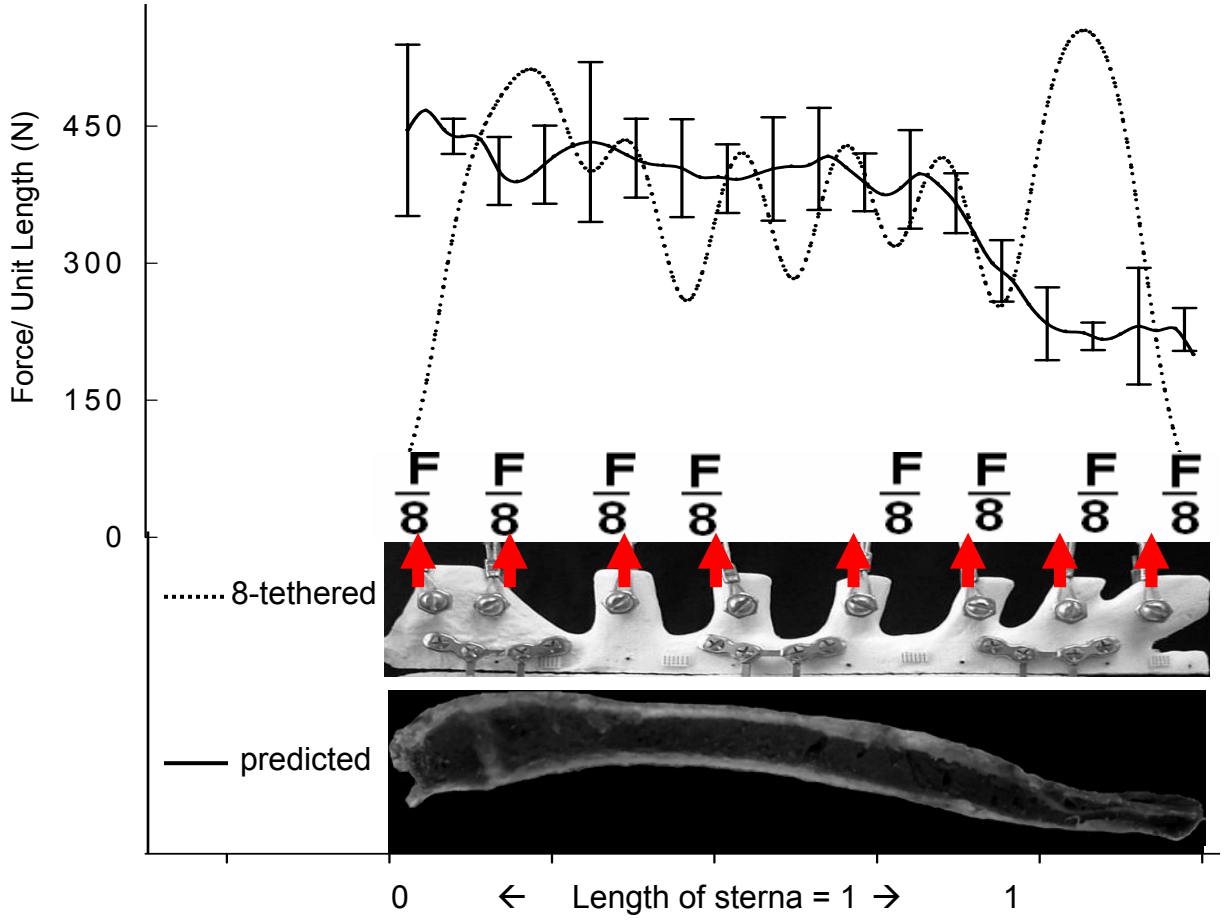


Figure 22: Estimation of distribution of forces across sternal midline *in vivo* (Pai, et al., Submitted).

6. Discussion

The success of a fixation device depends on its ability to withstand the distracting or separating forces that act along the sternal midline and to restrict micro-motion between the reapproximated bone halves. This micro-motion is detrimental to healing because it leads to callus formation (fibrous tissue that is weaker than native bone) (Chakkalakal, et al., 1999; Claes, et al., 2002; Yamaji, et al., 2001) and in severe cases may result in infection by preventing the open wound from closing. The results of this study present the first measurements of the forces acting on the sternal midline *in vivo* that may be applied to sternotomy models for the pre-clinical evaluation of a sternal fixation device.

6.1. Comparison of measured and estimated magnitude of force

As presented in the Background section, a simple mathematical model from previous methods (Casha, et al., 1999) used the Law of Laplace to estimate the lateral forces along the sternotomy midline by simplifying the chest to be a cylinder. The governing equation, T = RLP, yields that for a large patient during coughing (P=40 kPa) the force required to hold the sternum halves together may reach up to 1500 N. According to our data, for an approximate pressure of 35 kPa during a simulated cough the lateral force at the manubrium and xiphoid were only 44 N and 36 N respectively, yielding a total force across the sternum of 80 N. While it must be taken into account that a 60 kg (132lb) pig is not anatomically equivalent to a typical high-risk patient who may weigh over double the pig's weight, the difference in force magnitude is still surprising as the forces measured in this study are twenty times less than those predicted. Even previous *in vitro* loading regimes that have used smaller forces between 180 N and 400 N (Dasika, et al., 2003; Trumble, et al., 2002) seem excessive compared to the forces that might be extrapolated from our data for larger patients. These findings suggest that, unlike previous *in vitro* loading regimes, fixation device failure *in vivo* is not likely due to large catastrophic forces pulling the sternal halves apart. However, while the sternum may not be subjected to large respiratory loads, higher forces may result from lifting heavy objects, lying on

one's side, or other loading factors which were not investigated in this study and should be considered in future investigations (see Limitations).

6.2. Comparison of measured and estimated distribution of force

Our estimation of the distribution of forces along the sternal midline using the relative cortical bone densities of cadaver sterna and assuming Wolff's law predicts that the manubrium is loaded twice as heavily as the xiphoid. Thus it should not be surprising that we observed large separations at the xiphoid with this loading regime in our previous *in vitro* tests (Pai, et al., 2005) or that similar models that load the xiphoid heavily have made similar observations (Bruhin, et al., 2005; Dasika, et al., 2003). However, our current findings show the xiphoid to be subject to larger dynamic loads than the other locations, significantly during spontaneous and ventilated breathing at 20cm H_2O. Since the forces we measured are so small, it is likely that respiratory forces are not the primary determinant of sternal bone morphology and that the higher bone density we observed at the manubrium must compensate for other physiological functions (see Limitations). Thus, future studies should investigate possible non-respiratory loads placed on the manubrium that might account for its higher cortical bone density.

6.3. Comparison of measured and estimated direction of force

Many groups have previously assumed that forces in the lateral direction are dominant (Casha, et al., 1999; Losanoff, et al., 2004; Pai, et al., Submitted; Trumble, et al., 2002) and only a few *in vitro* device evaluation methods applied loads in all three coordinate directions (Cohen and Griffin, 2002). Our results show that while the lateral force direction appears to be predominant for both breathing and coughing, visible displacements and measurable forces were also noted in the cranial-caudal and dorsal-ventral directions, and the forces in all directions were on the same order of magnitude. Since shear forces are believed to delay healing due to shear motion between the bone halves (Augat, et al.,

2003), the forces in the cranial-caudal and dorsal-ventral directions may play a significant role during sternal healing. Consequently, our results indicate that future device testing should incorporate applied loads in multiple directions simultaneously for better physiological accuracy.

6.4. Potential of cadaveric models in future studies

There were few differences in forces along the midline between spontaneous versus ventilated breathing. This result was surprising because we expected there would be a difference between negative and positive pressure ventilation. Additionally, the effects of rigor mortis (death) and chemical fixation (embalming) were not significant. This lack of changes in force between treatments may be partially due to the fact that our specimens were preserved for a much shorter period than is typical of research-grade cadavers. Nonetheless, studies investigating the effects of death and fixation indicate that substantial changes in tissue properties occur within the time frame we employed (Van Ee, et al., 2000). Although the similarities between treatments might imply that ventilating a cadaver or dead animal model would simulate live spontaneous breathing, this conclusion should be made with caution since the forces were too small in general to distinguish between treatments. Thus, future investigations are still needed to evaluate the accuracy of cadaveric models for sternal device evaluation.

6.5. Limitations of the current study

The discrepancy between expected and measured force magnitude highlights the fact that respiratory forces may not be the primary cause of sternal closure failure. Our study is limited in this respect as we only studied respiratory forces in the supine position. Healthy people are likely to load their sternum when involved in upper-body exercise such as lifting weights or carrying a back-pack. However, patients who are recovering from median sternotomy are unlikely to strain themselves in these ways. In fact, they have the ability to control most forces on their sternum by not exerting

themselves; the only forces they cannot control are gravitational forces and respiratory forces, such as those induced by bouts of coughing. Hence, the maximal loads placed on a recovering patient's sternal midline are still likely to be induced by coughing. Future tests should consider investigating the effects of different postures such as prone versus supine or lying on one's side or chest.

Examining possible sources of error in our data, we ruled out a lack of sensitivity of our equipment as a source since we specifically chose transducers that are capable of measuring small changes in force (± 0.03 N for the 22.2 N transducers). Furthermore, the lack of statistical differences between our results was only partially due to the high animal-to-animal variability; these differences were taken into account by using pigs as a blocking factor in the ANOVA. One major source of error may have been the bulky design of our force measurement system. Since we were measuring much smaller forces than we had anticipated, it is likely that much of the variability in our data came from the way the force transducers were positioned. It was difficult to pre-tension the transducers or align the sternal edges perfectly with our instruments because of their size. While we prevented each of the force transducers from being loaded in any direction other than its specified direction by using ball-joints, and we used bone cement and metal plates to create a level platform to help align the transducers, it is possible that slight misalignments that were not immediately visible during placement on the sternum might have resulted in small differences in force. Since the total forces were so small, even small changes in force due to non-ideal positioning could lead to substantial variability. Hence, it is extremely likely that the high standard deviations we observed occurred due to small changes in transducer positioning. Additionally, it was difficult to control the vigor of both coughing forces. Although we attempted to apply uniform forces to the animal's abdomen for each valsalva cough, we did not take measurements to ensure the same force was applied and even with stimulation at the same voltage (40 V), there was little consistency between phrenic coughs. Despite these small inconsistencies that reduced the precision of our data, we remain confident about the accuracy of our data due to the high sensitivity of our force transducers. If a more ideal measurement

system were created, we believe that the key findings would be the same; that the respiratory forces on the sternum *in vivo* are very small.

6.6. Possible mechanism of sternal fixation device failure

Since the forces we measured are so small, it seems unlikely that the mechanism of failure of a fixation device is due to catastrophic failure from large loads or inadequate strength of the device itself. Instead, it is likely due to fatigue from progressive wear of the fixation device into the bone over long periods of cyclic loading. This fatigue likely occurs due to high stress concentrations at the bone-device interface, possibly created by pre-tensioning of wires or sharp screw threads. As the device progressively cuts through the bone, it allows for micro-motion. As an illustrative description of this mechanism, consider the dynamic forces acting on the sterna (forces pulling bisected sternal halves apart during respiration or coughing) where the following occurs: (1) instead of resisting the stresses placed on the bone by the device during each cycle, the device cuts through the bone until (2) it is also no longer forcing the two sternal halves to be held together so they can now move relative to each other thereby disrupting healing. For patients considered to be at high risk for complications because of weak bone quality (osteoporosis) or continual chronic coughing (emphysema), the creation of stress concentrations at the bone-device interface results in rapid degradation of the sternal wound. Like most elastic solids, fatigue on bone results from the application of cyclic stresses where the number of cycles to failure varies with the level of stress (Moore and Gibson, 2003; Pattin, et al., 1996). For example, it would take many cycles to failure at the low stresses caused by respiration whereas it would take fewer cycles to failure with the higher stresses caused by coughing. Note that failure in this context is not the catastrophic destruction of bone but rather the progressive failure at the bone-device interface leading to micro-motion at the midline that would be disruptive to sternal healing. Since healing of the sternum may take up to three months in some cases (Wu, et al., 2004), a fixation device might be considered successful if it could minimize micro-motion in a reapproximated

sternum for the number of stress cycles that would occur within this time period. Although the critical factor to control in wound healing is micro-motion, the causative factors that are important for consideration in the design of future sternal fixation devices will be to reduce stress concentrations while maintaining adequate purchase in weak bone.

6.7. Conclusions

The results of this paper demonstrate that while coughing produces the highest forces as might be expected, the magnitudes of respiratory and coughing forces along the sternal midline are much smaller than previously predicted. We also determined that the forces in the lateral direction are predominant, most notably at the xiphoid. These forces will need to be characterized for loading conditions other than respiration and coughing. In contrast to the loading regimes used in previous investigations of the efficacy fixation devices, it would appear that the success of a fixation device to reduce micro-motion between the reapproximated sternal halves depends less on withstanding large static or impulse forces and more on its ability to resist low magnitude cyclic loading. These findings present a paradigm shift in the way sternal fixation devices should be evaluated in the future. It is our belief that the development of more physiologically relevant device evaluation methods will reduce the need for further animal testing, decrease the time and costs associated with testing fixation devices, and thereby help improve the healthcare of patients undergoing open-heart surgery.

7. Recommendations

Based on the findings of this study, we recommend that future *in vitro* and numerical testing methods used to systematically test sternal fixation devices should incorporate small repetitive cyclic loads that induce fatigue of bone. This development in testing methods would require that each device be evaluated for how many cycles it would allow without causing "failure" (disruptive micro-motion at the sternal midline) in a realistic bone model for both breathing and coughing loading conditions. A device might be considered successful if the number of loading cycles to "failure" is greater than the number of cycles necessary for adequate healing. Although acceptable levels of micro-motion and healing time have been previously studied, we recommend further investigation into these parameters since they define the success or failure of a device evaluated with our proposed methods. Further, it is vital that a realistic bone model is used to characterize future loading regimes, particularly one that mimics poor bone quality, because fixation devices should be able to cater to patients in a worst-case scenario. To this effect, the device would need to prevent micro-motion for the highest expected forces. Currently these forces are believed to caused by coughing, as characterized in the present study; however, we also recommend that future studies investigate other possible forces that might act on a patient's sternum (e.g. from lying on their side).

We also recommend that numerical methods such as FEM should be used in the development of novel fixation devices because these methods are capable of predicting stress concentrations and could be used to design fixation systems that will minimize high stresses in bone of poor quality. Furthermore, they would provide a rapid initial evaluation of which devices and configurations would yield optimal fixation results before moving on to the above-described *in vitro* tests.

References

A.H.A. 2004. Heart disease and stroke statistics - 2005 update. American Heart Association.

Andriacchi, T., Schultz, A., Belytschko, T. and Galante, J. 1974. A model for studies of mechanical interactions between the human spine and rib cage. Journal of Biomechanics 7, 497-507.

Augat, P., Burger, J., Schorlemmer, S., Henke, T., Peraus, M. and Claes, L. 2003. Shear movement at the fracture site delays healing in a diaphyseal fracture model. Journal of Orthopaedic Research 21, 1011-7.

Baugmart, F. W. and Perren, S. M. 1994. Rationale for the design and use of pure titanium internal fixation plates. In: Harvey, J. P. and Games, R. F. (eds.), Clinical and laboratory performance of bone plates, astm stp 1217. American Society for Testing and Materials, pp. 25-33.

Becker, D. M., Lord, L. and Dobell, A. R. 1972. Techniques and pitfalls of anesthesia and thoracic surgery in the pig. Journal of Surgical Research 13, 215-9.

Bruhin, R., Stock, U. A., Drucker, J. P., Azhari, T., Wippermann, J., Albes, J. M., Hintze, D., Eckardt, S., Konke, C. and Wahlers, T. 2005. Numerical simulation techniques to study the structural response of the human chest following median sternotomy. Ann Thorac Surg 80, 623-30.

Bryan, A. J., Lamarra, M., Angelini, G. D., West, R. R. and Breckenridge, I. M. 1992. Median sternotomy wound dehiscence: A retrospective case control study of risk factors and outcome. Journal of the Royal College of Surgeons of Edinburgh 37, 305-8.

Casha, A. R., Yang, L. and Cooper, G. J. 1999. Measurement of chest wall forces on coughing with the use of human cadavers. J Thorac Cardiovasc Surg 118, 1157-8.

Casha, A. R., Yang, L., Kay, P. H., Saleh, M. and Cooper, G. J. 1999. A biomechanical study of median sternotomy closure techniques. European Journal of Cardio-thoracic Surgery 15, 365-9.

Centofanti, P., La Torre, M., Barbato, L., Verzini, A., Patane, F. and di Summa, M. 2002. Sternal closure using semirigid fixation with thermoreactive clips. Annals of Thoracic Surgery 74, 943-5.

Chakkalakal, D. A., Strates, B. S., Mashoof, A. A., Garvin, K. L., Novak, J. R., Fritz, E. D., Mollner, T. J. and McGuire, M. H. 1999. Repair of segmental bone defects in the rat: An experimental model of human fracture healing. Bone 25, 321-32.

Claes, L., Eckert-Hubner, K. and Augat, P. 2002. The effect of mechanical stability on local vascularization and tissue differentiation in callus healing. Journal of Orthopaedic Research 20, 1099-105.

Cohen, D. J. and Griffin, L. V. 2002. A biomechanical comparison of three sternotomy closure techniques. Annals of Thoracic Surgery 73, 563-8.

Cook, K. E., Makarewicz, A. J., Backer, C. L., Mockros, L. F., Przybylo, H. J., Crawford, S. E., Hernandez, J. M., Leonard, R. J. and Mavroudis, C. 1996. Testing of an intrathoracic artificial lung in a pig model. American Society of Artificial Internal Organs Journals 42, M604-9.

Cooper, P. R., Cohen, A., Rosiello, A. and Koslow, M. 1988. Posterior stabilization of cervical spine fractures and subluxations using plates and screws. Neurosurgery 23, 300-6.

Dasika, U. K., Trumble, D. R. and Magovern, J. A. 2003. Lower sternal reinforcement improves the stability of sternal closure. Annals of Thoracic Surgery 75, 1618-21.

De Troyer, A. 1989. The mechanism of the inspiratory expansion of the rib cage. J Lab Clin Med 114, 97-104.

De Troyer, A. and Decramer, M. 1985. Mechanical coupling between the ribs and sternum in the dog. Respir Physiol 59, 27-34.

De Troyer, A. and Estenne, M. 1988. Functional anatomy of the respiratory muscles. Clin Chest Med 9, 175-93.

De Troyer, A., Kirkwood, P. A. and Wilson, T. A. 2005. Respiratory action of the intercostal muscles. Physiol Rev 85, 717-56.

De Troyer, A. and Wilson, T. A. 1993. Sternum dependence of rib displacement during breathing. J Appl Physiol 75, 334-40.
Eich, B. S. and Heinz, T. R. 2000. Treatment of sternal nonunion with the dall-miles cable system. Plastic and Reconstructive Surgery 106, 1075-8.
El Oakley, R. M. and Wright, J. E. 1996. Postoperative mediastinitis: Classification and management. Annals of Thoracic Surgery 61, 1030-6.
Feher, G. 1996. Cyclopedia anatomicae. Black Dog & Leventhal Publishers, Inc.
Gottlieb, L. J., Pielet, R. W., Karp, R. B., Krieger, L. M., Smith, D. J., Jr. and Deeb, G. M. 1994. Rigid internal fixation of the sternum in postoperative mediastinitis. Archives of Surgery 129, 489-93.
Gray, H. 1918. Anatomy of the human body. Lea & Febiger.
Guidant. 2004. Sternotomy. In. http://www.medmovie.com/medialibrary/index.htm.
Hendrickson, S. C., Koger, K. E., Morea, C. J., Aponte, R. L., Smith, P. K. and Levin, L. S. 1996. Sternal plating for the treatment of sternal nonunion. Annals of Thoracic Surgery 62, 512-8.
Julian, O. C., Lopez-Belio, M., Dye, W. S., Javid, H. and Grove, W. J. 1957. The median sternal incision in intracardiac surgery with extracorporeal circulation; a general evaluation of its use in heart surgery. Surgery 42, 753-61.
Karp, R. 1996. Advances in cardiac surgery. Mosby - Year Book Inc.
Loop, F. D., Lytle, B. W., Cosgrove, D. M., Mahfood, S., McHenry, M. C., Goormastic, M., Stewart, R. W., Golding, L. A. and Taylor, P. C. 1990. J. Maxwell chamberlain memorial paper. Sternal wound complications after isolated coronary artery bypass grafting: Early and late mortality, morbidity, and cost of care. Annals of Thoracic Surgery 49, 179-86; discussion 186-7.
Loring, S. H. and Woodbridge, J. A. 1991. Intercostal muscle action inferred from finite-element analysis. Journal of Applied Physiology 70, 2712-8.
Losanoff, J. E., Collier, A. D., Wagner-Mann, C. C., Richman, B. W., Huff, H., Hsieh, F., Diaz-Arias, A. and Jones, J. W. 2004. Biomechanical comparison of median sternotomy closures. Annals of Thoracic Surgery 77, 203-9.
Losanoff, J. E., Foerst, J. R., Huff, H., Richman, B. W., Collier, A. D., Hsieh, F. H., Lee, S. and Jones, J. W. 2002. Biomechanical porcine model of median sternotomy closure. J Surg Res 107, 108-12.
Malvern, L. E. 1969. Introduction to the mechanics of a continuous medium. Prentice-Hall.
Mayba, II. 1985. Non-union of fractures of the sternum. The Journal of Bone and Joint Surgery 67, 1091-3.
McGregor, W. E., Payne, M., Trumble, D. R., Farkas, K. M. and Magovern, J. A. 2003. Improvement of sternal closure stability with reinforced steel wires. Ann Thorac Surg 76, 1631-4.
McGregor, W. E., Trumble, D. R. and Magovern, J. A. 1999. Mechanical analysis of midline sternotomy wound closure. Journal of Thoracic and Cardiovascular Surgery 117, 1144-50.
Mier, A., Brophy, C., Estenne, M., Moxham, J., Green, M. and De Troyer, A. 1985. Action of abdominal muscles on rib cage in humans. J Appl Physiol 58, 1438-43.
Molina, J. E., Lew, R. S. and Hyland, K. J. 2004. Postoperative sternal dehiscence in obese patients: Incidence and prevention. Annals of Thoracic Surgery 78, 912-7; discussion 912-7.
Moore, T. L. and Gibson, L. J. 2003. Fatigue of bovine trabecular bone. J Biomech Eng 125, 761-8.
Ouellette, A., Kato, S., Nakamura, K., Latta, L. L. and Burkhalter, W. E. 1994. Mechanical evaluation of internal and external fixation for metacarpal fractures. In: Harvey, J. P. and Games, R. F. (eds.), Clinical and laboratory performance of bone plates, astm stp 1217. American Society for Testing and Materials, pp. 95-102.
Ozaki, W., Buchman, S. R., Iannettoni, M. D. and Frankenburg, E. P. 1998. Biomechanical study of sternal closure using rigid fixation techniques in human cadavers. Annals of Thoracic Surgery 65, 1660-5.

Pai, S., Gunja, N. J., Dupak, E. L., McMahon, N. L., Lalikos, J., Dunn, R. M., Francalancia, N., Pins, G. D. and Billiar, K. L. Submitted. A mechanical study of rigid plate configurations for sternal fixation. Journal of Biomechanics.

Pai, S., Gunja, N. J., Dupak, E. L., McMahon, N. L., Lalikos, J., Dunn, R. M., Roth, T., Francalancia, N., Pins, G. D. and Billiar, K. L. 2005. *In vitro* comparison of wire and plate fixation for midline sternotomies. Annals of Thoracic Surgery 80, 962-968.

Pattin, C. A., Caler, W. E. and Carter, D. R. 1996. Cyclic mechanical property degradation during fatigue loading of cortical bone. J Biomech 29, 69-79.

Popesko, P. 1977. Atlas of topographical anatomy of the domestic animals. W.B. Saunders Company.

Roberts, S. B. and Chen, P. H. 1970. Elastostatic analysis of the human thoracic skeleton. Journal of Biomechanics 3, 527-45.

Sargent, L. A., Seyfer, A. E., Hollinger, J., Hinson, R. M. and Graeber, G. M. 1991. The healing sternum: A comparison of osseous healing with wire versus rigid fixation. Annals of Thoracic Surgery 52, 490-4.

Smoot, E. C. and Weiman, D. 1998. Paramedian sternal bone plate reinforcement and wiring for difficult sternotomy wounds. Annals of Plastic Surgery 41, 464-7.

Song, D. H., Lohman, R. F., Renucci, J. D., Jeevanandam, V. and Raman, J. 2004. Primary sternal plating in high-risk patients prevents mediastinitis. European Journal of Cardiothoracic Surgery 26, 367-72.

Stahle, E., Tammelin, A., Bergstrom, R., Hambreus, A., Nystrom, S. O. and Hansson, H. E. 1997. Sternal wound complications--incidence, microbiology and risk factors. European Journal of Cardiothoracic Surgery 11, 1146-53.

Stoney, W. S., Alford, W. C., Jr., Burrus, G. R., Frist, R. A. and Thomas, C. S., Jr. 1978. Median sternotomy dehiscence. Annals of Thoracic Surgery 26, 421-6.

Sundaram, S. H. and Feng, C. C. 1977. Finite element analysis in the human thorax. Journal of Biomechanics 10, 505-16.

Tavilla, G., van Son, J. A., Verhagen, A. F. and Lacquet, L. K. 1991. Modified robicsek technique for complicated sternal closure. Annals of Thoracic Surgery 52, 1179-80.

Trumble, D. R. and Magovern, J. A. 2004. Comparison of dog and pig models for testing substernal cardiac compression devices. American Society for Artificial Internal Organs Journal 50, 188-92.

Trumble, D. R., McGregor, W. E. and Magovern, J. A. 2002. Validation of a bone analog model for studies of sternal closure. Annals of Thoracic Surgery 74, 739-44; discussion 745.

Van Ee, C. A., Chasse, A. L. and Myers, B. S. 2000. Quantifying skeletal muscle properties in cadaveric test specimens: Effects of mechanical loading, postmortem time, and freezer storage. J Biomech Eng 122, 9-14.

White, T. 2000. Human osteology. Academic Press.

Wilke, H. J., Krischak, S. and Claes, L. E. 1996. Formalin fixation strongly influences biomechanical properties of the spine. Journal of Biomechanics 29, 1629-31.

Wu, L. C., Renucci, J. and Song, D. H. 2004. Rigid-plate fixation for the treatment of sternal nonunion. J Thorac Cardiovasc Surg 128, 623-4.

Yamaji, T., Ando, K., Wolf, S., Augat, P. and Claes, L. 2001. The effect of micromovement on callus formation. Journal of Orthopaedic Science 6, 571-5.

Appendix A: Data from pig one

Pig 1 compiled data (values in lb)	**M Lateral**		**Longitudinal**		**Vertical**		**X Lateral**	
	Static	*Dynamic*	*Static*	*Dynamic*	*Static*	*Dynamic*	*Static*	*Dynamic*
Live								
Spontaneous, unretracted	6.76	0.16	-0.71	0.18	4.46	0.09	2.29	0.54
Spontaneous, 1/2 retracted	7.02	0.24	-0.93	0.54	4.29	0.34	2.45	1.19
Spontaneous, fully retracted	7.11	0.23	-0.97	0.52	4.48	0.40	2.32	1.18
Ventilated-1/2 retracted 20cmH20	6.96	0.21	-0.96	0.56	4.39	0.26	2.62	0.91
Ventilated-fully retracted	7.02	0.23	-0.84	0.42	4.57	0.27	2.66	0.77
Ventilated fast-fully retracted	7.05	0.16	-0.91	0.43	4.61	0.21	2.82	0.69
Vent 500ml-full retrac 20cmH2O	7.27	0.04	-0.89	0.05	4.71	0.04	3.26	0.12
Coughing, fully retracted	7.25	0.16	-0.87	0.28	4.57	0.19	3.30	0.68
Dead								
Ventilated 30cmH2O, 300ml 25bpm	7.05	0.06	-0.73	0.02	4.46	0.08	3.06	0.10
Ventilated 30cmH20, 400ml 20bpm	7.10	0.04	-0.73	0.14	4.50	0.07	3.09	0.14
Embalmed								
Ventilated 30cmH2O, 200ml 20bpm	7.75	0.07	-0.72	0.26	4.67	0.14	3.45	0.08
Ventilated 40cmH20, 300ml 20bpm	7.75	0.10	-0.80	0.37	4.66	0.19	3.46	0.11
Ventilated 30cmH2O, 200ml 25bpm	7.71	0.11	-0.72	0.33	4.67	0.15	3.40	0.11

AVERAGE PEAK

	M Lateral	Longitudi	Vertical	X Lateral
L-S unretracted	0.16	0.18	0.09	0.54
L-S 1/2 retracted	0.24	0.54	0.34	1.19
L-S fully retracted	0.23	0.52	0.40	1.00
L-V 1/2 retracted 20cmH2O	0.21	0.56	0.26	0.91
L-V fully retracted	0.23	0.42	0.27	0.77
L-V fast-fully retracted	0.16	0.43	0.21	0.69
L-V 20cm H2O 500ml	0.04	0.05	0.04	0.12
L-phrenic cough	0.16	0.28	0.19	0.68
D-V 30cmH2O, 300ml	0.06	0.02	0.08	0.10
D-V 30cmH2O, 400ml	0.04	0.14	0.07	0.14
E-V 30cmH20 200ml 20bpm	0.07	0.26	0.14	0.08
E-V 40cmH20 300ml	0.10	0.37	0.19	0.11
E-V 30cmH20 200ml 25bpm	0.11	0.33	0.15	0.11

AVERAGE BASE

	M Lateral	Longitudi	Vertical	X Lateral
L-S unretracted	6.76	0.71	4.46	2.29
L-S 1/2 retracted	7.02	0.93	4.29	2.45
L-S fully retracted	7.11	0.97	4.48	2.32
L-V 1/2 retracted	6.96	0.96	4.39	2.62
L-V fully retracted	7.02	0.84	4.57	2.66
L-V fast-fully retracted	7.05	0.91	4.61	2.82
L-V 20cm H2O 500ml	7.27	0.89	4.71	3.26
L-phrenic cough	7.25	0.87	4.57	3.30
D-V 30cmH2O, 300ml	7.05	0.73	4.46	3.06
D-V 30cmH2O, 400ml	7.10	0.73	4.50	3.09
E-V 30cmH20 200ml 20bpm	7.75	0.72	4.67	3.45
E-V 40cmH20 300ml	7.75	0.80	4.66	3.46
E-V 30cmH20 200ml 25bpm	7.71	0.72	4.67	3.40

	pressure (psi)		pressure (kPa)	
	static	dynamic	static	dynamic
L-V 20cm H2O 500ml	0.33	0.05	2.30	0.32
L-phrenic cough	0.32	0.12	2.18	0.80
D-V 30cmH2O, 300ml	0.77	0.04	5.29	0.28
D-V 30cmH2O, 400ml	0.74	0.03	5.11	0.24

	pressure±SD (psi)	
static	0.54	0.25
dynamic	0.25	0.43

Appendix B: Data from pig two

Pig 2 compiled data (lb)	M Lateral		Longitudinal		Vertical		X Lateral	
	Static	*Dynamic*	*Static*	*Dynamic*	*Static*	*Dynamic*	*Static*	*Dynamic*
Live 4 Directions								
Spontaneous, unretracted	-2.84	0.09	-0.53	0.34	0.52	0.11	4.72	0.48
Spontaneous, 1/2 retracted	0.57	0.57	-0.17	0.38	0.43	0.09	4.70	0.80
Phrenic nerve cough	-1.72	1.02	0.52	1.37	0.44	0.37	5.07	2.72
Valsalva cough	-1.81	0.29	0.55	0.66	0.49	0.30	5.02	0.60
Ventilated 20cmH2O 450ml	-1.89	0.15	0.24	0.34	0.42	0.08	5.36	0.54
Live 2 Directions								
Ventilated 20cmH2O 450ml	-0.01	0.03					-0.11	0.11
Ventilated 30cmH2O 480ml	-0.05	0.11					-0.09	0.21
Valsalva cough 20cmH2O	-0.05	1.41					-0.09	0.86
Dead 2 Directions								
Ventilated 20cmH2O 325ml	0.03	0.03					-0.05	0.07
Ventilated 30cmH2O 400ml	-0.10	0.16					0.02	0.05
Valsalva cough 20cmH2O	-0.13	0.05					0.06	0.23
Embalmed 2 Directions								
Ventilated 20cmH2O 320ml	0.03	0.10					0.19	0.10
Ventilated 30cmH2O 350ml	0.05	0.19					0.19	0.20
Refridgerated 2 Directions								
Ventilated 20cmH2O 180ml	0.77	0.24					0.18	0.16
Ventilated 30cmH2O 220ml	0.69	0.48					0.17	0.29
Valsalva cough 20cmH2O	0.62	0.25					0.21	0.14
Ventilated 40cmH2O 300ml	0.57	0.37					0.16	0.47

AVERAGE PEAK

	M Lateral	Longitudi	Vertical	X Lateral
L-S, unretracted	0.09	0.34	0.11	0.48
L-S, 1/2 retracted	0.57	0.38	0.09	0.80
L-S, phrenic cough	1.02	1.37	0.37	2.72
L-S, valsalva cough	0.29	0.66	0.30	0.60
L-V, 20cmH2O 450ml	0.15	0.34	0.08	0.54
L-V, 20cmH2O 450ml	0.03			0.11
L-V, 30cmH2O 480ml	0.11			0.21
L-V, cough 20cmH2O	1.41			0.86
D-V, 20cmH2O 325ml	0.03			0.07
D-V, 30cmH2O 400ml	0.16			0.05
D-V, cough 20cmH2O	0.05			0.23
E-V, 20cmH2O 320ml	0.10			0.10
E-V, 30cmH2O 350ml	0.19			0.20
R-V, 20cmH2O 180ml	0.24			0.16
R-V, 30cmH2O 220ml	0.48			0.29
R-V, cough 20cmH2O	0.25			0.14
R-V, 40cmH2O 300ml	0.37			0.47

AVERAGE BASE

	M Lateral	Longitudi	Vertical	X Lateral
L-S, unretracted	2.84	0.53	0.52	4.72
L-S, 1/2 retracted	0.57	0.17	0.43	4.70
L-S, phrenic cough	1.72	0.52	0.44	5.07
L-S, valsalva cough	1.81	0.55	0.49	5.02
L-V, 20cmH2O 450ml	1.89	0.24	0.42	5.36
L-V, 20cmH2O 450ml	0.01			0.11
L-V, 30cmH2O 480ml	0.05			0.09
L-V, cough 20cmH2O	0.05			0.09
D-V, 20cmH2O 325ml	0.03			0.05
D-V, 30cmH2O 400ml	0.10			0.02
D-V, cough 20cmH2O	0.13			0.06
E-V, 20cmH2O 320ml	0.03			0.19
E-V, 30cmH2O 350ml	0.05			0.19
R-V, 20cmH2O 180ml	0.77			0.18
R-V, 30cmH2O 220ml	0.69			0.17
R-V, cough 20cmH2O	0.62			0.21
R-V, 40cmH2O 300ml	0.57			0.16

Appendix C: Data from pig three

Pig 3 compiled data (lb)	M Lateral		Longitudinal		Vertical		X Lateral	
	Static	*Dynamic*	*Static*	*Dynamic*	*Static*	*Dynamic*	*Static*	*Dynamic*
Live 4 Directions	N/A, ANIMAL DIED DURING STERNOTOMY							
Live 2 Directions	N/A, ANIMAL DIED DURING STERNOTOMY							
Just Dead 2 Directions								
Ventilated 20cmH2O 400ml	0.09	0.03					-0.02	0.01
Ventilated 30cmH2O 420ml	0.11	0.03					-0.01	0.03
Ventilated 40cmH2O 600ml	0.11	0.06					-0.02	0.06
Valsalva cough 20cmH2O	0.10	0.02					0.00	0.15
Dead 2 Directions								
Ventilated 20cmH2O 350ml	0.21	0.02					-0.05	0.02
Ventilated 30cmH2O 400ml	0.15	0.04					-0.01	0.02
Ventilated 40cmH2O 560ml	0.15	0.09					-0.01	0.03
Valsalva cough 20cmH2O	0.14	0.11					0.00	0.25
Embalmed 2 Directions								
Ventilated 20cmH2O 100ml	0.28	0.00					0.07	0.01
Ventilated 30cmH2O 200ml	0.26	0.01					0.08	0.01
Ventilated 40cmH2O 250ml	0.26	0.00					0.08	0.02
Ventilated 60cmH2O 325ml	0.26	0.02					0.11	0.04
Valsalva cough 20cmH2O	0.26	0.25					0.08	0.48
Refridgerated 2 Directions								
Ventilated 20cmH2O 50ml	0.57	0.02					0.22	0.01
Ventilated 30cmH2O 100ml	0.54	0.01					0.25	0.01
Ventilated 40cmH2O 200ml	0.52	0.01					0.28	0.01
Ventilated 60cmH2O 320ml	0.51	0.02					0.28	0.05
Valsalva cough 20cmH2O	0.51	0.16					0.19	0.12

AVERAGE PEAK

	M Lateral	Longitudir	Vertical	X Lateral
JD-V 20cmH2O 400ml	0.03			0.01
JD-V 30cmH2O 420ml	0.03			0.03
JD-V 40cmH2O 600ml	0.06			0.06
JD-V cough 20cmH2O	0.02			0.15
D-V 20cmH2O 350ml	0.02			0.02
D-V 30cmH2O 400ml	0.04			0.02
D-V 40cmH2O 560ml	0.09			0.03
D-V cough 20cmH2O	0.11			0.25
E-V 20cmH2O 100ml	0.00			0.01
E-V 30cmH2O 200ml	0.01			0.01
E-V 40cmH2O 250ml	0.00			0.02
E-V 60cmH2O 325ml	0.02			0.04
E-V cough 20cmH2O	0.25			0.48
R-V 20cmH2O 50ml	0.02			0.01
R-V 30cmH2O 100ml	0.01			0.01
R-V 40cmH2O 200ml	0.01			0.01
R-V 60cmH2O 320ml	0.02			0.05
R-V cough 20cmH2O	0.16			0.12

AVERAGE BASE

	M Lateral	Longitudir	Vertical	X Lateral
JD-V 20cmH2O 400ml	0.09			-0.02
JD-V 30cmH2O 420ml	0.11			-0.01
JD-V 40cmH2O 600ml	0.11			-0.02
JD-V cough 20cmH2O	0.10			0.00
D-V 20cmH2O 350ml	0.21			-0.05
D-V 30cmH2O 400ml	0.15			-0.01
D-V 40cmH2O 560ml	0.15			-0.01
D-V cough 20cmH2O	0.14			0.00
E-V 20cmH2O 100ml	0.28			0.07
E-V 30cmH2O 200ml	0.26			0.08
E-V 40cmH2O 250ml	0.26			0.08
E-V 60cmH2O 325ml	0.26			0.11
E-V cough 20cmH2O	0.26			0.08
R-V 20cmH2O 50ml	0.57			0.22
R-V 30cmH2O 100ml	0.54			0.25
R-V 40cmH2O 200ml	0.52			0.28
R-V 60cmH2O 320ml	0.51			0.28
R-V cough 20cmH2O	0.51			0.19

Appendix D: Data from pig four

Pig 4 compiled data (lb)	M Lateral		Longitudinal		Vertical		X Lateral	
	Static	*Dynamic*	*Static*	*Dynamic*	*Static*	*Dynamic*	*Static*	*Dynamic*
Live 4 Directions								
Spontaneous, unretracted	5.39	0.15	2.16	0.17	-1.60	0.05	-0.18	1.26
Spontaneous, 1/2 retracted	5.68	0.15	2.05	0.39	-1.72	0.22	-0.27	1.61
LS + Phrenic nerve cough	5.72	28.34	2.02	4.44	-1.92	6.21	1.77	20.76
LS + Valsalva cough	6.17	0.65	0.75	0.89	-2.86	0.94	1.79	0.61
Ventilated 20cmH2O 500ml	5.82	0.20	2.11	0.88	-2.05	0.24	0.69	1.35
t 20cmH2O 500ml+ valsalva	5.83	0.83	2.20	0.85	-1.93	0.56	1.89	0.35
Ventilated 30cmH2O 650ml	5.83	0.16	2.06	0.61	-2.03	0.12	1.79	0.56

	M Lateral		Pressure		X Lateral	
Live 2 Directions	*Static*	*Dynamic*	*Static*	*Dynamic*	*Static*	*Dynamic*
Ventilated 20cmH2O 500ml	0.08	0.33			-0.54	0.66
Ventilated 30cmH2O 700ml	0.13	0.13			-0.24	0.40
Valsalva cough 20cmH2O	0.20	0.39			-0.68	0.77
Phrenic cough 20cmH2O	0.21	0.72			-0.09	0.88
+Ventilated 20cmH2O 500ml	0.39	0.06	4.51	1.09	-28.18	N/A
PT+Spontaneous+phrenic	0.38	3.12	5.07	1.26	-28.82	N/A
Dead 2 Directions						
Ventilated 20cmH2O 200ml	0.38	0.05	0.06	0.16	-28.82	N/A
Ventilated 30cmH2O 400ml	0.37	0.08	0.08	0.23	-28.82	N/A
Valsalva cough 20cmH2O	0.38	0.37	5.58	0.31	-28.82	N/A
Embalmed 2 Directions						
Ventilated 20cmH2O 200ml	0.62	0.10	0.08	0.18	-28.82	N/A
Ventilated 30cmH2O 350ml	0.50	0.17	0.09	0.24	-28.82	N/A
Valsalva cough 20cmH2O	0.55	0.45	5.85	0.66	-28.82	N/A
Ventilated 40cmH2O 525ml	0.48	0.26	0.09	0.32	-28.82	N/A
Refridgerated 2 Directions						
Ventilated 20cmH2O 90ml	1.37	0.13			0.43	0.20
Ventilated 30cmH2O 150ml	1.21	0.18			0.24	0.33
Valsalva cough 20cmH2O	1.40	1.05			0.52	0.64
Ventilated 40cmH2O 250ml	1.22	0.32			0.21	0.44
Ventilated 60cmH2O 650ml	1.15	0.68			0.19	0.67

AVERAGE PEAK

	M Lateral	Longitudi	Vertical	X Lateral
L-S, unretracted	0.15	0.17	0.05	1.26
L-S, 1/2 retracted	0.15	0.39	0.22	1.61
L-S, phrenic cough	28.34	4.44	6.21	20.76
L-S, valsalva cough	0.65	0.89	0.94	0.61
L-V, 20cmH2O 500ml	0.20	0.88	0.24	1.35
L-V, 20cmH2O + valsalva	0.83	0.85	0.56	0.35
L-V, 30cmH2O 650ml	0.16	0.61	0.12	0.56

	M Lateral	Pressure	X Lateral
L-V, 20cmH2O 500ml	*0.33*		0.66
L-V, 30cmH2O 700ml	*0.13*		0.40
L-V, valsalva 20cmH2O	*0.39*		0.77
L-V, phrenic 20cmH2O	*0.72*		0.88
L-V, 20cmH2O 500ml +PT	*0.06*	1.09	N/A
L-S, phrenic +PT	*3.12*	1.26	N/A
D-V, 20cmH2O 200ml	0.05	0.16	N/A
D-V, 30cmH2O 400ml	0.08	0.23	N/A
D-V, cough 20cmH2O	0.37	0.31	N/A

	Pressure±SD(psi)	
static	2.38	2.75
range	0.06	5.85
dynamic	0.49	0.42
range	0.16	1.26
	Pressure±SD(kPa)	
static	16.39	18.97
range	0.39	40.31
dynamic	3.40	2.86
range	1.11	8.67

E-V, 20cmH2O 200ml	0.10	0.18	N/A
E-V, 30cmH2O 350ml	0.17	0.24	N/A
E-V, cough 20cmH2O	0.45	0.66	N/A
E-V, 40cmH2O 525ml	0.26	0.32	N/A
R-V, 20cmH2O 90ml	0.13		0.20
R-V, 30cmH2O 150ml	0.18		0.33
R-V, cough 20cmH2O	1.05		0.64
R-V, 40cmH2O 250ml	0.32		0.44
R-V, 60cmH2O 650ml	0.68		0.67

AVERAGE BASE

	M Lateral	Longitudi	Vertical	X Lateral
L-S, unretracted	5.39	2.16	-1.60	-0.18
L-S, 1/2 retracted	5.68	2.05	-1.72	-0.27
L-S, phrenic cough	5.72	2.02	-1.92	1.77
L-S, valsalva cough	6.17	0.75	-2.86	1.79
L-V, 20cmH2O 500ml	5.82	2.11	-2.05	0.69
L-V, 20cmH2O + valsalva	5.83	2.20	-1.93	1.89
L-V, 30cmH2O 650ml	5.83	2.06	-2.03	1.79

	M Lateral Pressure		X Lateral
L-V, 20cmH2O 500ml	0.08		-0.54
L-V, 30cmH2O 700ml	0.13		-0.24
L-V, valsalva 20cmH2O	0.20		-0.68
L-V, phrenic 20cmH2O	0.21		-0.09
L-V, 20cmH2O 500ml +PT	0.39	4.51	-28.18
L-S, phrenic +PT	0.38	5.07	-28.82
D-V, 20cmH2O 200ml	0.38	0.06	-28.82
D-V, 30cmH2O 400ml	0.37	0.08	-28.82
D-V, cough 20cmH2O	0.38	5.58	-28.82
E-V, 20cmH2O 200ml	0.62	0.08	-28.82
E-V, 30cmH2O 350ml	0.50	0.09	-28.82
E-V, cough 20cmH2O	0.55	5.85	-28.82
E-V, 40cmH2O 525ml	0.48	0.09	-28.82
R-V, 20cmH2O 90ml	1.37		0.43
R-V, 30cmH2O 150ml	1.21		0.24
R-V, cough 20cmH2O	1.40		0.52
R-V, 40cmH2O 250ml	1.22		0.21
R-V, 60cmH2O 650ml	1.15		0.19

Appendix E: Statistics in SigmaStat (Two-way ANOVA with Tukey HSD post-hoc analysis)

Question 1: For each treatment, is there a predominant direction?

Dependent Variable: L-S unretracted

Normality Test:	Passed	(P = 0.235)
Equal Variance Test:	Passed	(P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	2	0.0690	0.0345	0.633	0.563
Direction	3	0.888	0.296	5.431	0.038
Residual	6	0.327	0.0545		
Total	11	1.283	0.117		

The difference in the mean values among the different levels of Direction is greater than would be expected by chance after allowing for effects of differences in Animal. There is a statistically significant difference (P = 0.038). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Animal : 0.0502
Power of performed test with alpha = 0.0500: for Direction : 0.588

Least square means for Animal :

Group	Mean
Pig 1	0.242
Pig 2	0.254
Pig 3	0.409

Std Err of LS Mean = 0.117

Least square means for Direction :

Group	Mean
M Lateral	0.131
Longitudinal	0.230
Vertical	0.0824
X Lateral	0.764

Std Err of LS Mean = 0.135

All Pairwise Multiple Comparison Procedures (Tukey Test):
Comparisons for factor: Direction

Comparison	Diff of Means	p	q	P	P<0.050
X Lateral vs. Vertical	0.681	4	5.056	0.044	Yes
X Lateral vs. M Lateral	0.633	4	4.694	0.059	No
X Lateral vs. Longitudinal	0.534	4	3.965	0.109	Do Not Test
Longitudinal vs. Vertical	0.147	4	1.092	0.865	No
Longitudinal vs. M Lateral	0.0983	4	0.730	0.952	Do Not Test
M Lateral vs. Vertical	0.0488	4	0.362	0.994	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Dependent Variable: L-S 1/2 retract

Normality Test:	Passed	(P = 0.172)
Equal Variance Test:	Passed	(P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	2	0.0416	0.0208	0.288	0.759
Direction	3	1.792	0.597	8.274	0.015
Residual	6	0.433	0.0722		
Total	11	2.267	0.206		

The difference in the mean values among the different levels of Direction is greater than would be expected by chance after allowing for effects of differences in Animal. There is a statistically significant difference (P = 0.015). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Animal : 0.0502
Power of performed test with alpha = 0.0500: for Direction : 0.812

Least square means for Animal :

Group	Mean
Pig 1	0.576
Pig 2	0.461
Pig 3	0.593

Std Err of LS Mean = 0.134

Least square means for Direction :

Group	Mean
M Lateral	0.320
Longitudinal	0.439
Vertical	0.216
X Lateral	1.199

Std Err of LS Mean = 0.155

All Pairwise Multiple Comparison Procedures (Tukey Test):
Comparisons for factor: Direction

Comparison	Diff of Means	p	q	P	P<0.050
X Lateral vs. Vertical	0.983	4	6.335	0.017	Yes
X Lateral vs. M Lateral	0.879	4	5.663	0.027	Yes
X Lateral vs. Longitudinal	0.760	4	4.897	0.050	No
Longitudinal vs. Vertical	0.223	4	1.438	0.747	No
Longitudinal vs. M Lateral	0.119	4	0.766	0.945	Do Not Test
M Lateral vs. Vertical	0.104	4	0.671	0.962	Do Not Test

Dependent Variable: L-Phrenic cough

Normality Test:	Passed	(P = 0.136)
Equal Variance Test:	Passed	(P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	2	531.553	265.777	6.152	0.035
Direction	3	143.707	47.902	1.109	0.416
Residual	6	259.221	43.204		
Total	11	934.481	84.953		

The difference in the mean values among the different levels of Animal is greater than would be expected by chance after allowing for effects of differences in Direction. There is a statistically significant difference (P = 0.035). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Animal : 0.592
Power of performed test with alpha = 0.0500: for Direction : 0.0615

Least square means for Animal :

Group	Mean	SEM
Pig 1	0.328	3.286
Pig 2	1.367	3.286
Pig 3	14.937	3.286

Least square means for Direction :

Group	Mean	SEM
M Lateral	9.839	3.795
Longitudinal	2.030	3.795
Vertical	2.255	3.795
X Lateral	8.051	3.795

All Pairwise Multiple Comparison Procedures (Tukey Test):
Comparisons for factor: Direction

Comparison	Diff of Means	p	q	P	P<0.050
M Lateral vs. Longitudinal	7.810	4	2.058	0.514	No
M Lateral vs. Vertical	7.584	4	1.998	0.536	Do Not Test
M Lateral vs. X Lateral	1.788	4	0.471	0.986	Do Not Test
X Lateral vs. Longitudinal	6.022	4	1.587	0.691	Do Not Test
X Lateral vs. Vertical	5.796	4	1.527	0.713	Do Not Test
Vertical vs. Longitudinal	0.226	4	0.0595	1.000	Do Not Test

Dependent Variable: L-V, 20cmH2O

Normality Test:	Passed	(P = 0.630)
Equal Variance Test:	Passed	(P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	2	0.303	0.151	4.613	0.061
Direction	3	1.158	0.386	11.772	0.006
Residual	6	0.197	0.0328		
Total	11	1.658	0.151		

The difference in the mean values among the different levels of Direction is greater than would be expected by chance after allowing for effects of differences in Animal. There is a statistically significant difference (P = 0.006). To isolate which group(s) differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.0500: for Animal : 0.443
Power of performed test with alpha = 0.0500: for Direction : 0.938

Least square means for Animal :

Group	Mean
Pig 1	0.486
Pig 2	0.278
Pig 3	0.667

Std Err of LS Mean = 0.0906

Least square means for Direction :

Group	Mean
M Lateral	0.186
Longitudinal	0.592
Vertical	0.195
X Lateral	0.934

Std Err of LS Mean = 0.105

All Pairwise Multiple Comparison Procedures (Tukey Test):
Comparisons for factor: Direction

Comparison	Diff of Means	p	q	P	P<0.050

X Lateral vs. M Lateral	0.748	4	7.151	0.009	Yes
X Lateral vs. Vertical	0.739	4	7.065	0.010	Yes
X Lateral vs. Longitudinal	0.341	4	3.264	0.198	No
Longitudinal vs. M Lateral	0.406	4	3.887	0.116	No
Longitudinal vs. Vertical	0.397	4	3.801	0.125	Do Not Test
Vertical vs. M Lateral	0.00897	4	0.0858	1.000	Do Not Test

Dependent Variable: L-valsalva

Normality Test: Passed (P = 0.485)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.190	0.190	5.476	0.101
Direction	3	0.0939	0.0313	0.904	0.532
Residual	3	0.104	0.0346		
Total	7	0.388	0.0554		

Power of performed test with alpha = 0.0500: for Animal : 0.313
Power of performed test with alpha = 0.0500: for Direction : 0.0537

Least square means for Animal :

Group	Mean
Pig 2	0.463
Pig 3	0.770

Std Err of LS Mean = 0.0931

Least square means for Direction :

Group	Mean
M Lateral	0.468
Longitudinal	0.774
Vertical	0.619
X Lateral	0.605

Std Err of LS Mean = 0.132

Question 2: For each direction, is there a change in force with treatment?

Dependent Variable: M Lateral

Normality Test: Failed (P < 0.050)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	2	126.543	63.271	0.980	0.428
Treatment	3	208.579	69.526	1.077	0.427
Residual	6	387.251	64.542		
Total	11	722.373	65.670		

Power of performed test with alpha = 0.0500: for Animal : 0.0502
Power of performed test with alpha = 0.0500: for Treatment : 0.0583

Least square means for Animal :

Group	Mean
Pig 1	0.191
Pig 2	0.458
Pig 3	7.209

Std Err of LS Mean = 4.017

Least square means for Treatment :

Group	Mean
L-S, unretracted	0.131
L-S, 1/2 retracted	0.320
L-S, phrenic cough	9.839
L-V, 20cmH2O	0.186

Std Err of LS Mean = 4.638

Dependent Variable: Longitudinal

Normality Test: Passed (P = 0.095)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	2	2.605	1.302	1.138	0.381
Treatment	3	6.027	2.009	1.755	0.255
Residual	6	6.868	1.145		
Total	11	15.499	1.409		

Power of performed test with alpha = 0.0500: for Animal : 0.0632
Power of performed test with alpha = 0.0500: for Treatment : 0.134

Least square means for Animal :

Group	Mean
Pig 1	0.392
Pig 2	0.606
Pig 3	1.470

Std Err of LS Mean = 0.535

Least square means for Treatment :

Group	Mean
L-S, unretracted	0.230
L-S, 1/2 retracted	0.439
L-S, phrenic cough	2.030
L-V, 20cmH2O	0.593

Std Err of LS Mean = 0.618

Dependent Variable: Vertical

Normality Test:	Failed	(P < 0.050)
Equal Variance Test:	Passed	(P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	2	5.937	2.969	1.011	0.418
Treatment	3	9.867	3.289	1.120	0.412
Residual	6	17.621	2.937		
Total	11	33.426	3.039		

Power of performed test with alpha = 0.0500: for Animal : 0.0512
Power of performed test with alpha = 0.0500: for Treatment : 0.0626

Least square means for Animal :

Group	Mean
Pig 1	0.220
Pig 2	0.160
Pig 3	1.681

Std Err of LS Mean = 0.857

Least square means for Treatment :

Group	Mean
L-S, unretracted	0.0824
L-S, 1/2 retracted	0.216
L-S, phrenic cough	2.255
L-V, 20cmH2O	0.195

Std Err of LS Mean = 0.989

Dependent Variable: X Lateral

Normality Test:	Failed	(P < 0.050)
Equal Variance Test:	Passed	(P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	2	74.059	37.030	1.297	0.340
Treatment	3	113.262	37.754	1.323	0.351
Residual	6	171.261	28.544		
Total	11	358.582	32.598		

Power of performed test with alpha = 0.0500: for Animal : 0.0787
Power of performed test with alpha = 0.0500: for Treatment : 0.0842

Least square means for Animal :

Group	Mean
Pig 1	0.830
Pig 2	1.135
Pig 3	6.246

Std Err of LS Mean = 2.671

Least square means for Treatment :

Group	Mean
L-S, unretracted	0.764
L-S, 1/2 retracted	1.199
L-S, phrenic cough	8.051
L-V, 20cmH2O	0.934

Std Err of LS Mean = 3.085

Dependent Variable: M Lateral

Normality Test:	Failed	(P < 0.050)
Equal Variance Test:	Passed	(P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	3	126.608	42.203	0.654	0.609
Treatment	4	243.009	60.752	0.941	0.500
Residual	6	387.251	64.542		
Total	13	730.373	56.183		

Power of performed test with alpha = 0.0500: for Animal : 0.0505
Power of performed test with alpha = 0.0500: for Treatment : 0.0506

Least square means for Animal :

Group	Mean	SEM
Pig 1	-1.121	4.400
Pig 2	-0.854	4.400
Pig2	5.535	10.778
Pig 3	5.897	3.593

Least square means for Treatment :

Group	Mean	SEM
L-S, unretracted	1.188	5.500
L-S, 1/2 retracted	1.377	5.500
L-S, phrenic cough	10.896	5.500
L-V, 20cmH2O	1.243	5.500
L-S, valsalva cough	-2.884	6.811

Dependent Variable: Longitudinal

Normality Test:	Failed	(P < 0.050)
Equal Variance Test:	Passed	(P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	3	2.631	0.877	0.766	0.553
Treatment	4	6.298	1.575	1.376	0.346
Residual	6	6.868	1.145		
Total	13	15.529	1.195		

Power of performed test with alpha = 0.0500: for Animal : 0.0505
Power of performed test with alpha = 0.0500: for Treatment : 0.0924

Least square means for Animal :

Group	Mean	SEM
Pig 1	0.275	0.586
Pig 2	0.490	0.586
Pig2	1.126	1.435
Pig 3	1.353	0.478

Least square means for Treatment :

Group	Mean	SEM
L-S, unretracted	0.334	0.732
L-S, 1/2 retracted	0.544	0.732
L-S, phrenic cough	2.135	0.732
L-V, 20cmH2O	0.697	0.732
L-S, valsalva cough	0.345	0.907

Dependent Variable: Vertical

Normality Test:	Failed	(P < 0.050)
Equal Variance Test:	Passed	(P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	3	6.139	2.046	0.697	0.587
Treatment	4	10.310	2.578	0.878	0.529
Residual	6	17.621	2.937		
Total	13	33.636	2.587		

Power of performed test with alpha = 0.0500: for Animal : 0.0505
Power of performed test with alpha = 0.0500: for Treatment : 0.0506

Least square means for Animal :

Group	Mean	SEM
Pig 1	0.0710	0.939
Pig 2	0.0116	0.939
Pig2	0.897	2.299
Pig 3	1.533	0.766

Least square means for Treatment :

Group	Mean	SEM
L-S, unretracted	0.172	1.173
L-S, 1/2 retracted	0.305	1.173
L-S, phrenic cough	2.345	1.173
L-V, 20cmH2O	0.285	1.173
L-S, valsalva cough	0.0328	1.453

Dependent Variable: X Lateral

Normality Test:	Failed	(P < 0.050)
Equal Variance Test:	Passed	(P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	3	74.059	24.686	0.865	0.509
Treatment	4	138.685	34.671	1.215	0.395
Residual	6	171.261	28.544		
Total	13	366.373	28.183		

Power of performed test with alpha = 0.0500: for Animal : 0.0505
Power of performed test with alpha = 0.0500: for Treatment : 0.0738

Least square means for Animal :

Group	Mean	SEM
Pig 1	-0.298	2.926
Pig 2	0.00765	2.926
Pig2	5.112	7.168
Pig 3	5.118	2.389

Least square means for Treatment :

Group	**Mean**	**SEM**
L-S, unretracted	1.639	3.658
L-S, 1/2 retracted	2.074	3.658
L-S, phrenic cough	8.927	3.658
L-V, 20cmH2O	1.809	3.658
L-S, valsalva cough	-2.025	4.529

Question 3: For each location (m-lateral/ x-lateral), is there a change in force with treatment?

Dependent Variable: M Lateral

Normality Test: Passed (P = 0.658)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.00588	0.00588	0.386	0.578
Treatment	3	0.0296	0.00985	0.647	0.635
Residual	3	0.0457	0.0152		
Total	7	0.0811	0.0116		

Power of performed test with alpha = 0.0500: for Animal : 0.0521
Power of performed test with alpha = 0.0500: for Treatment : 0.0537

Least square means for Animal :

Group	Mean
Pig 2	0.0996
Pig 3	0.154

Std Err of LS Mean = 0.0617

Least square means for Treatment :

Group	Mean
L-V, 20cmH2O	0.183
D-V, 20cmH2O	0.0404
E-V, 20cmH2O	0.0987
R-V, 20cmH2O	0.185

Std Err of LS Mean = 0.0872

Dependent Variable: X Lateral

Normality Test: Passed (P = 0.077)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.0855	0.0855	1.328	0.455
Treatment	3	0.0449	0.0150	0.233	0.870
Residual	1	0.0644	0.0644		
Total	5	0.245	0.0491		

Power of performed test with alpha = 0.0500: for Animal : 0.0991
Power of performed test with alpha = 0.0500: for Treatment : 0.0940

Least square means for Animal :

Group	Mean	SEM
Pig 2	0.111	0.127
Pig 3	0.404	0.220

Least square means for Treatment :

Group	Mean	SEM
L-V, 20cmH2O	0.387	0.179
D-V, 20cmH2O	0.217	0.284
E-V, 20cmH2O	0.244	0.284
R-V, 20cmH2O	0.183	0.179

Dependent Variable: M Lateral

Normality Test: Passed (P = 0.683)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.0185	0.0185	1.843	0.268
Treatment	3	0.0569	0.0190	1.891	0.307
Residual	3	0.0301	0.0100		
Total	7	0.106	0.0151		

Power of performed test with alpha = 0.0500: for Animal : 0.102
Power of performed test with alpha = 0.0500: for Treatment : 0.113

Least square means for Animal :

Group	Mean
Pig 2	0.235
Pig 3	0.138

Std Err of LS Mean = 0.0501

Least square means for Treatment :

Group	Mean
L-V, 30cmH2O	0.121
D-V, 30cmH2O	0.119
E-V, 30cmH2O	0.180
R-V, 30cmH2O	0.326

Std Err of LS Mean = 0.0708

Dependent Variable: X Lateral

Normality Test: Passed (P = 0.077)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.0130	0.0130	2.529	0.357

Treatment	3	0.0264	0.00881	1.716	0.499
Residual	1	0.00513	0.00513		
Total	5	0.0728	0.0146		

Power of performed test with alpha = 0.0500: for Animal : 0.121
Power of performed test with alpha = 0.0500: for Treatment : 0.106

Least square means for Animal :

Group	Mean	SEM
Pig 2	0.188	0.0358
Pig 3	0.302	0.0621

Least square means for Treatment :

Group	Mean	SEM
L-V, 30cmH2O	0.304	0.0507
D-V, 30cmH2O	0.108	0.0801
E-V, 30cmH2O	0.257	0.0801
R-V, 30cmH2O	0.309	0.0507

Dependent Variable: M Lateral

Normality Test: Passed (P = 0.458)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.00180	0.00180	0.00404	0.955
Treatment	2	0.482	0.241	0.543	0.648
Residual	2	0.889	0.444		
Total	5	1.373	0.275		

Power of performed test with alpha = 0.0500: for Animal : 0.0578
Power of performed test with alpha = 0.0500: for Treatment : 0.0592

Least square means for Animal :

Group	Mean
Pig 2	0.569
Pig 3	0.604

Std Err of LS Mean = 0.385

Least square means for Treatment :

Group	Mean
L-V, cough	0.897
D-V, cough	0.211
R-V, cough	0.651

Std Err of LS Mean = 0.471

Dependent Variable: X Lateral

Normality Test: Passed (P = 0.125)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Col 12	1	0.0407	0.0407	0.454	0.623
Col 13	2	0.230	0.115	1.279	0.530
Residual	1	0.0898	0.0898		
Total	4	0.424	0.106		

Power of performed test with alpha = 0.0500: for Animal : 0.0926
Power of performed test with alpha = 0.0500: for Treatment : 0.0990

Least square means for Animal :

Group	Mean	SEM
Pig 2	0.411	0.173
Pig 3	0.613	0.245

Least square means for Treatment :

Group	Mean	SEM
L-V, cough	0.816	0.212
D-V, cough	0.326	0.335
R-V, cough	0.394	0.212

Question 4: For each location (m-lateral/ x-lateral), is there a change in force at different ventilation pressures (within treatment groups of live, dead, embalmed, and refrigerated)?

Dependent Variable: M Lateral

Normality Test: Passed (P = 0.474)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.0808	0.0808	0.334	0.622
Treatment	2	0.743	0.372	1.538	0.394
Residual	2	0.483	0.242		
Total	5	1.307	0.261		

Power of performed test with alpha = 0.0500: for Animal : 0.0578
Power of performed test with alpha = 0.0500: for Treatment : 0.0829

Least square means for Animal :

Group	Mean
Pig 2	0.516
Pig 3	0.284

Std Err of LS Mean = 0.284

Least square means for Treatment :

Group	Mean
L-V, 20cmH2O	0.183
L-V, 30cmH2O	0.121
L-V, cough	0.897

Std Err of LS Mean = 0.348

Dependent Variable: X Lateral

Normality Test: Passed (P = 0.365)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.0670	0.0670	1.286	0.374
Treatment	2	0.302	0.151	2.901	0.256
Residual	2	0.104	0.0521		
Total	5	0.473	0.0946		

Power of performed test with alpha = 0.0500: for Animal : 0.0703
Power of performed test with alpha = 0.0500: for Treatment : 0.140

Least square means for Animal :

Group	Mean
Pig 2	0.396
Pig 3	0.608

Std Err of LS Mean = 0.132

Least square means for Treatment :

Group	Mean
L-V, 20cmH2O	0.387
L-V, 30cmH2O	0.304
L-V, cough	0.816

Std Err of LS Mean = 0.161

Dependent Variable: M Lateral

Normality Test: Passed (P = 0.463)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.0113	0.0113	0.519	0.546
Treatment	2	0.0293	0.0146	0.672	0.598
Residual	2	0.0436	0.0218		
Total	5	0.0842	0.0168		

Power of performed test with alpha = 0.0500: for Animal : 0.0578
Power of performed test with alpha = 0.0500: for Treatment : 0.0592

Least square means for Animal :

Group	Mean
Pig 2	0.0800
Pig 3	0.167

Std Err of LS Mean = 0.0852

Least square means for Treatment :

Group	Mean
D-V, 20cmH2O	0.0403
D-V, 30cmH2O	0.119
D-V, cough	0.211

Std Err of LS Mean = 0.104

Dependent Variable: M Lateral

Normality Test: Passed (P = 0.197)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.000148	0.000148	1.126	0.481
Treatment	1	0.00659	0.00659	50.230	0.089
Residual	1	0.000131	0.000131		
Total	3	0.00686	0.00229		

Power of performed test with alpha = 0.0500: for Animal : 0.0951
Power of performed test with alpha = 0.0500: for Treatment : 0.412

Least square means for Animal :

Group	Mean
Pig 2	0.145
Pig 3	0.133

Std Err of LS Mean = 0.00810

Least square means for Treatment :

Group	Mean
E-V, 20cmH2O	0.0986
E-V, 30cmH2O	0.180

Std Err of LS Mean = 0.00810

Dependent Variable: M Lateral

Normality Test: Passed (P = 0.657)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.0155	0.0155	0.130	0.742
Treatment	3	0.231	0.0772	0.649	0.635
Residual	3	0.357	0.119		
Total	7	0.604	0.0862		

Power of performed test with alpha = 0.0500: for Animal : 0.0521
Power of performed test with alpha = 0.0500: for Treatment : 0.0537

Least square means for Animal :

Group	Mean
Pig 2	0.333
Pig 3	0.421

Std Err of LS Mean = 0.172

Least square means for Treatment :

Group	Mean
R-V, 20cmH2O	0.185
R-V, 30cmH2O	0.326
R-V, cough	0.651
R-V, 40cmH2O	0.345

Std Err of LS Mean = 0.244

Dependent Variable: X Lateral

Normality Test:	Passed	(P = 0.503)
Equal Variance Test:	Passed	(P = 1.000)

Source of Variation	DF	SS	MS	F	P
Animal	1	0.0377	0.0377	1.254	0.344
Treatment	3	0.0852	0.0284	0.944	0.518
Residual	3	0.0902	0.0301		
Total	7	0.213	0.0304		

Power of performed test with alpha = 0.0500: for Animal : 0.0671
Power of performed test with alpha = 0.0500: for Treatment : 0.0537

Least square means for Animal :

Group	Mean
Pig 2	0.267
Pig 3	0.405

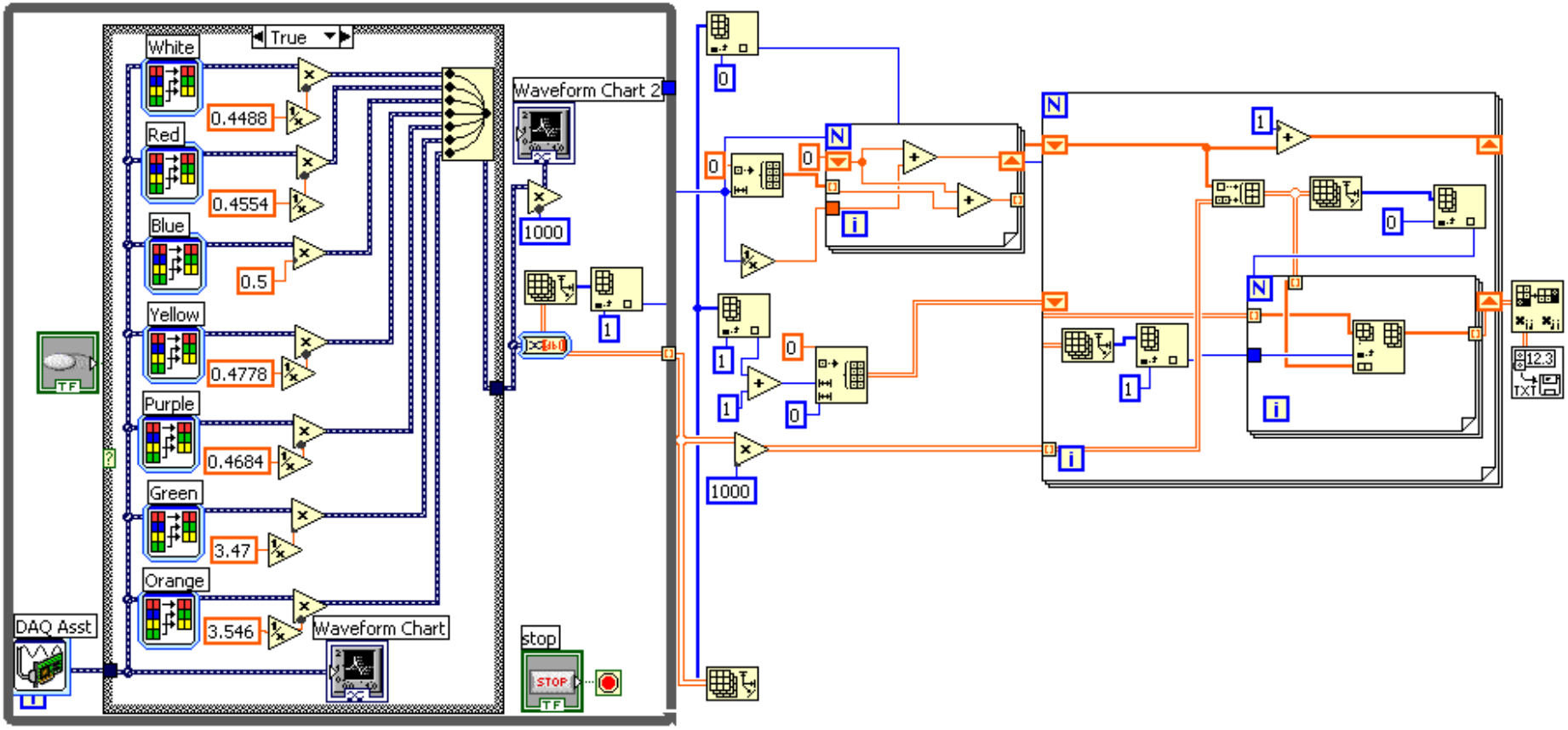
Std Err of LS Mean = 0.0867

Least square means for Treatment :

Group	Mean
R-V, 20cmH2O	0.183
R-V, 30cmH2O	0.309
R-V, cough	0.394
R-V, 40cmH2O	0.458

Std Err of LS Mean = 0.123

Appendix F: Block diagram of LabView program used during data acquisition



Appendix G: Matlab program used to separate static and dynamic forces

```
%Created by Paul Branche, Modified by Russ Dresher, 6/23/05, Modified by Shruti Pai, 7/18/05
%Calculates the average amplitude and average lower peak of a respiratory waveform

%LOADS TEXT FILE + SMOOTHS SIGNAL
clear;                                  %clears memory
clc;                                    %clears command window
load r25_modified.txt                   %load file
X = r25_modified(:,2);                  %assigns short variable name, looks at specified data column
[B,A] = butter (2,.05);                 %2nd order LPF with 25 Hz cutoff
Y = filter(B,A,X);
range = (100:3500);                     %range of data points to be examined for peaks (first 100 data
                                        %points omitted due to transient)

%POSITIVE PEAK DETECTION LOOP
count = 1;
for u = 2:1:(length(Y))-1;
  r = diff(Y);                          %first derivative
%positive peak detection
  if r(u - 1) > 0 & r(u) <= 0 & Y(u) >0.51    %detects change in sign of slope
      u;                                %shows indeces of peak
      peaks_index(count) = u;           %stores indeces in temp variable
      count = count + 1;
  end
end
%positive spline calculation
x = peaks_index;
c = Y(peaks_index);
xx = [1 :length(Y)];
yy = spline(x,c,xx);

%NEGATIVE PEAK DETECTION LOOP
counts = 1;
for uu = 2:1:(length(Y))-1;
%negative peak detection
  if r(uu - 1) < 0 & r(uu) >= 0 & Y(uu) <0.51  %Y(uu) is amplitude, must adjust boundary for each signal
      uu;
      peaks_indexs(counts) = uu;
      counts = counts + 1;
  end
end
%negative spline calculation
x1 = peaks_indexs;
c1 = Y(peaks_indexs);
xx1 = [1 : length(Y)];
yy1 = spline(x1,c1,xx1);

%PLOTS WAVEFORM WITH NEGATIVE AND POSITIVE ENVELOPES (splines)
figure(1)
plot(xx,yy,'b')
hold on;
plot(Y,'r')
hold on;
plot(xx,mean(yy(range)),'k')            %plots entire signal (positive envelope)
hold on;
plot(xx,mean(yy1(range)),'k')           %plots entire signal (negative envelope)
hold on;
```

```
plot(xx1,yy1,'g')
hold off;

%TWO METHODS TO CALCULATE AVERAGE AMPLITUDE

%1) substract mean of negative envelope from mean of positive envelope
dynamic_force = mean(yy(range)) - mean(yy1(range))

%2) calculate average peak to peak values
countt = 1;
for t=1:1:length(xx)
   individual_peak(countt) = yy(t) - yy1(t);
   countt = countt + 1;
end
check_dynamic_force = mean(individual_peak(range))  % mean omits first 100 data points due to transient

%CALCULATE AVERAGE PEAK VALUES
static_force = mean(yy1(range))              %lower peak
maximum_peak = mean(yy(range))               %upper peak
```

Appendix H: IACUC protocol

APPLICATION TO USE VERTEBRATE ANIMALS IN RESEARCH OR INSTRUCTION
UMMS Institutional Animal Care and Use Committee

Docket No. A-1687

Principal Investigator (Must be UMMS Faculty	**Name and Degree**	Kristen L. Billiar, Ph.D.
	Faculty Title	Adjunct Assistant Professor of Surgery
	Department \ Division or Company Name	Department of Surgery, UMMS Department of Biomedical Engineering, WPI (Primary Appointment)
	Mailing Address Building	100 Institute Road, Worcester, MA 01602 Salisbury Labs
	e-mail Address	kbilliar@wpi.edu
	FAX No. Telephone No.	508-831-5541 **Pager #:** 781-953-2549 **Office:** 508-831-5384 **Home:** 508-793-1946
Co-Investigator	**Name and Degree**	Raymond Dunn, M.D.
	Faculty Title	Chief, Department of Surgery
	Department \ Division or Company Name	Department of Surgery, UMMS
Project Title	Mechanical analysis of forces exerted on the sternum *in vivo* for the design of an accurate sternal fixation testing device	

X **New** ____ **3 Year Renewal** ____ **Major Amendment (state changes in a cover letter)** ____ **FDA-Mandated Level 3 (21CFR610)**

USE OF THIS APPLICATION FORM

- **DO NOT change formatting of this document. Type in non-shaded areas only. Forms submitted to IACUC MUST include shading. If attachments are used, they should be minimum and not replace answers to any section.**
- **In preparing this form, please refer to the *Institutional Animal Care and Use Committee Instruction Book.***
- **If you have questions regarding the completion of this form, please contact the IACUC office (508.856.5384) or one of the Dept. of Animal Medicine veterinarians at (508.856.3151) for guidance.**
- **Please note that upon request the University may be required by law to release a copy of this application to the public.**

Signature of Department Chair	**Date**	**Name (Please Print or Type)**
_____		Raymond Dunn, M.D.

For IACUC USE ONLY:

	Date	**Recommend Approval**	**Recommend Modification or Clarification**	**Initials**
Veterinary Review	_____			
IACUC Reviewer	_____			

Approved: _____ **DATE**

_____ **Chair/Vice Chair, IACUC**

PARTICIPATING PERSONNEL (including PI and Co-Investigator)

Identify all personnel expected to be manipulating and/or euthanizing animals at the time of this application.

Name	UMMS Phone	Emergency Phone	How many years of experience do you have with the proposed techniques in this animal model?	Completed Occupational Health Form
Kristen Billiar, Ph.D.	N/A	781-953-2549	0	X YES NO
Shruti Pai	N/A	215-870-5410	0	X YES NO
Raymond Dunn, M.D.	508-856-5299		1	X YES NO
Nicola Francalancia,	508-334-7828		1	X YES NO
Timothy Roth	508-856-2380		0	X YES NO
Adam Saltman	508-334-3278		20	X YES NO
Suzanne Wheeler	508-856-3644		10	X YES NO
Heather Strom	508-856-1729		0	X YES NO
Helena Zec	N/A	774-253-9595	0	X YES NO

Primary Contact Person	**Name**	Kristen Billiar
	Telephone No.	**Office** 508-831-5384 **Home** 508-793-1946
	Mailing Address Building	100 Institute Road, Worcester, MA 01602 Salisbury Labs

NOTE: Before other new personnel perform any procedures, a written amendment request must be submitted to and approved by the IACUC. Training and written guidelines on animal handling and basic procedures are available from the Dept. of Animal Medicine (telephone: 508.856.3151).

Signatures of all personnel listed above (including PI and Co-Investigator)

I have been given an opportunity to read this proposed research study and understand my responsibilities with regard to the care of animals involved.

Signature: _____ **Name (please type):** Kristen Billiar

Signature: _____ **Name (please type):** Raymond Dunn

Signature: _____ **Name (please type):** Nicola Francalancia

Signature: _____ **Name (please type):** Adam Saltman

Signature: _____ **Name (please type):** Timothy Roth

Signature: _____ **Name (please type):** Suzanne Wheeler

Signature: _____ **Name (please type):** Shruti Pai

Signature: _____ **Name (please type):** Heather Strom

Signature: _____ **Name (please type):** Helena Zec

A. OBJECTIVES OF PROPOSED RESEARCH OR INSTRUCTION

A1. Check the box below that describes the type of animal use being proposed.

X Basic Research | Service (Cores, Sentinels, etc.) | Testing (Biologicals, Toxicity, etc.)

Field Research | Instruction or Training | Applied Research | Other_____

A2: Lay Summary: In clear, concise, *non-technical*, language (i.e., that could be understood by someone at a high school level), summarize the background and specific aims of your studies involving animals.

Sternal fixation has been studied in cadavers and live animals, yet the magnitude, direction, and distribution of forces on the sternum in vivo and how they are altered by median sternotomy are not known. The purpose of this pilot study is to determine the validity of cadaveric models.

Although cadavers have been proposed as anatomically appropriate models, cadaveric soft tissue, whether "fresh" or embalmed, is less compliant than live tissue and hence may shield the actual loading that would be experienced by the sternum in a living patient. Alternatively, live animals such as pigs are considered more physiologically accurate, yet they are not anatomically equivalent to a human. We propose to quantify the forces acting on the sternum in pigs before and after sacrifice and embalming to investigate the effects of death and chemical fixation. If substantial changes in the magnitude or distribution of loads occur due to death and fixation, we will use the loading data from the living animals as a first approximation of the loads in a human patient.

Specific aims of the project are to determine the:

1) forces acting on the sternum acting at each rib strut in a living porcine model under normal respiration and coughing.

2) distribution of force on the sternum in living, fresh, and embalmed pigs to evaluate the validity of using fresh or embalmed cadavers in lieu of live animals in future biomechanical studies.

A3: Briefly explain the relevance of the proposed research or instruction to human or animal health and/or to the advancement of scientific knowledge.

Median sternotomy, a routine surgical approach used in open-heart surgery, has a 0.5 to 2.5% complication rate associated with its traditional use of stainless steel sutures for sternal closure (Stahle et al., 1997) translating to approximately 15,000 cases of poor sternal healing and dehiscence in the US every year (NCHS, 2003). Sternal dehiscence leads to discomfort, mediastinitis, osteomyelitis, and is associated with a 10-40% mortality rate (Tavilla et al., 1991). Improving the mechanical stability of sternal fixation devices using rigid plates, as is done for all other bone fractures, appears to facilitate more rapid sternal healing and decrease the likelihood of complications (Ozaki, 1998; Sargent, 1991; Song, 2004). Currently, the number and placement of plates are chosen by the surgeon intuitively.

Our goal is to determine the optimal sternal fixation configuration to obtain maximum stability of fixation of the sternum following midline sternotomy. We recently completed an engineering investigation of sternal fixation (in preparation) which indicates that plates are superior to wires. Our testing model consisted of a custom machined pulley system that would replicate the lateral forces across the sternum during normal breathing. However, in analyzing our data (and data from other groups), we determined that a more physiologically relevant testing model is needed to make valid comparisons between plate configurations in vitro. Specifically, an even lateral distribution of forces along the sternum appears to yield unrealistically large distraction of a) the xiphoid region and b) the posterior region. To avoid these limitations, sternal fixation has been studied in cadavers and live animals, however, optimization studies must be conducted in vitro to limit variability and cost.

To develop a better model, measurements of the forces on the sternum need to be made in intact chest cavities subjected to realistic breathing and coughing loads. For our studies, we propose to 1) determine the validity of cadavers for measurements of in vivo forces, 2) measure the forces acting on the sternum in animal and/or cadavers, 3) design a test system which applies a controlled and accurate distribution of forces in vitro, and 4) compare the stability of various fixation plate types and configurations using this device.

DATABASE SEARCHES

In the space below, document that you have searched databases

1. **To determine that you are not unnecessarily duplicating previous experiments, AND**
2. **To determine that alternatives to animal use are either not available or not appropriate.**

Dates of Searches: 07/01/04, 07/10/04, 07/26/04, 02/07/05, 02/09/05, 04/13/05

Name of the database(s) you searched: Science Direct, PUBMed/Medline, UMMS veterinarians (508.856.3151), Virtual Library of Biosciences http://golgi.harvard.edu/biopages.html, Virtual Library of Veterinary Medicine http://netvet.wustl.edu/vetmed.htm, Altweb http://www.jhsph.edu/~altweb/

Years Covered by Searches: 1965 to present

Keywords Searched: Chest Wall Mechanics, Thoracic Surgery, Sternal Fixation, Pig, Sternum, Animal Model, Animal alternatives, In Vitro Model

Other Sources of Information: Worcester Polytechnic Institute Database

B. FUNDING INFORMATION

1. SOURCE(S) OF FUNDING: Name the funding source(s) and then check the appropriate box(es).

Sponsor or Company Name:	Johnson & Johnson		**UMMS Account Number:**		Speedtype: 110286 Fund: 23323 DeptID: W824000014 Program: B03 P/G: S82050082000000
Federal Government		**Industry Sponsor**		**UMMS Programs**	
State or Other Government		**Other Private**		**Department**	

2. STATUS OF FUNDING: Is funding pending or approved?	**Pending**		**Approved**	X
3. Has or will this protocol undergo peer review as part of a grant application and be evaluated for scientific merit and experimental design:	**Yes** X		**No**	

C. RATIONALE FOR USING ANIMALS AND ALTERNATIVES TO THE USE OF ANIMALS

C1: Briefly explain why animals are required for your studies.
We have already conducted a study *in vitro* to determine how different closure devices affect the stability of the sternum after post-surgery fixation but found that the results were strongly dependent on the way the force was applied. Since the methods we used were only an approximation of what happens *in vivo* and it is not possible to measure these forces in humans as it requires invasive surgery, we require animals to study the actual force distribution on a living sternum. Although several studies have used cadavers as a better indicator of *in vivo* conditions, we are not sure that this is physiologically accurate. Hence in order to validate whether using cadavers is acceptable, we also need to first conduct tests in a living model to see the difference in tissue compliance after fixation. The proposed study is non-survival.

C2: Briefly explain why the species you propose to use is/are the most appropriate.
Pigs have been shown to be a good large animal model of the human thorax and have been used extensively in cardiothoracic studies. *(American Society of Artificial Internal Organs Journals, 1996; 42; p. M604-9 and 2004; 50; p. 188-92)*

C3: Describe the steps you have taken to reduce the usage of animals and to minimize the lethality of procedures in your experiments (e.g., using cell culture, computer simulations, or non-living models; doing pilot studies, using most specific assays possible.
We will first be conducting pilot studies in vitro using cadaveric pig thoraxes from previous studies to determine exactly how we will conduct each stage of the experiments. Thus we will have the opportunity to refine our methods to ensure satisfactory

NUMBER OF ANIMALS REQUESTED FOR 3 YEARS: PAIN / DISTRESS LEVEL

D1: List the number of animals you will use over the 3-year duration of this protocol. All animals must be accounted for, including embryos and neonates. The total for each row should equal the sum of the values in that row.

Species	Number in Pain / Distress Level C	Number in Pain / Distress Level D	Number in Pain / Distress Level E	Species Total For 3 Years
Yorkshir	0	3	0	3

Pain / Distress level indicates maximum pain or distress level to be experienced by animal(s):
C = negligible;
D = pain / distress relieved by appropriate drug use;
E = pain / distress not relieved by appropriate drug use. See IACUC Instruction Book for definitions and examples

D2: If you have animals in category E, use this space to provide a description of the procedures producing pain or distress, and list the reasons why pain-relieving drugs cannot or will not be used to relieve pain or distress. If pain/distress relief would interfere with test results, justify why that is true.

N/A

D3: If there are federal guidelines that *require* that you use a category E procedure, then use this space to cite the agency, CFR title, number and specific section.

N/A

E. JUSTIFICATION OF THE NUMBER OF ANIMALS REQUESTED

NIH rules require that animal use must be kept to the minimum consistent with a sound scientific outcome. Please use the space below to document that the number of animals requested is appropriate for the goals of the experiments.

E1: Write a brief description of experimental design. In a table, show all of the experimental groups and the number of animals per group. Be sure that the totals in the table match the totals shown in Section D (Number of Animals Requested).

Three test groups (treatments) will be used, namely Live, Dead, and Embalmed. The same three pigs will be tested after each treatment so although n=3 for each group, the total number of pigs used in the experiment will also be three. For each treatment the pigs intact chest cavities will be subjected to realistic breathing and coughing loads and measurements of the forces on the sternum will be made using force and pressure transducers. The force transducers will be implanted **along the midline of the sternum after a routing sternotomy by a surgeon using custom fixture plates that will be screwed into the bone.** Pressure transducers will be placed on underside of the sternum. The experiments will be conducted in the Animal Medicine main operating rooms for the first treatment (Live); after euthanasia the animals will be moved to the necropsy facility in Animal Medicine to measure forces for the remaining treatments (Dead and Embalmed).

Treatment (Group)	No. of animals used (pigs)	Total No. of Animals
Live	3	
Dead	3	3
Embalmed	3	

E2: Describe, in general terms, the statistical tests required for the study.

Repeated measures ANOVA will be used to determine the effect of treatment (live, dead, embalmed) on the average force magnitude measured and the distribution of forces along the length of the sternum.

F. USE OF ANIMALS OUTSIDE OF ANIMAL FACILITIES.

F1. Will animals be used in areas, e.g., laboratories, outside one of the general animal facilities? (A Level, BioTech II, Shriver main facility, MBL main facility, BNRI 1st floor, LRB 1st floor, Rose Gordon Facility) If "No", proceed to Section G.	**YES**	**X NO**
If "yes", list the building and room number(s) where animals will be housed or used outside the animal facility.		

Indicate which of the following procedures will be used in the protocol:

___ Breeding ___ Fluid Collection ___ Non-Surgical Procedure _X__ Non-Survival Surgery ___ Survival Surgery ___ Tissue Harvesting ___ Other:

Will animals be held, housed, and/or used in study areas outside of the animal facility for <u>more than 12 hours?</u> If "Yes", in the space below list the building and room number(s), and justify scientifically the need to hold animals for over 12 hours.	**YES**	**X NO**

F2. Use this space to describe how you will transport animals between the animal facility and the study area.

F3. Is a patient procedural area to be used for animal studies? If "Yes", use the space below to provide room number and/or location of patient area.	**YES**	**X NO**

F4. If "Yes", describe any special animal transport or facility procedures that will be followed to assure health and safety of both animals and patients.

G. ANIMAL SPECIFICS

G1. Describe the age/weight, sex, and source of each animal species/strain.

SPECIES / STRAIN	AGE / WEIGHT	SEX	*VENDOR
Yorkshire Pigs	100lb-200lb	F	Parsons Farm

G2. If any of your animals have special needs, use the space below to list needs for special handling or housing.

G3. Specify what parameters you will assess to ensure that the animals are healthy before your experiments begin. Check all boxes that apply.

Activity	X	**Appearance**	X	**Appetite**	X	**Behavior**	X
Excreta	X	**Respiratory Pattern**	X	**Temperature**	X	**Weight**	X
Laboratory tests or other observations (specify)							

H. EXPERIMENTAL PROCEDURES: ALL STUDIES EXCEPT SURVIVAL SURGERY

H1: In this space, describe, in narrative form, all procedures to be carried out on living animals from initial contact to euthanasia. A sequential list of activities involving live animals is usually the clearest and most efficient format. Do NOT include details of *in vitro* procedures.

Three treatments will be studied using the same 3 animals per group hence each animal will undergo the same steps.

Animal Prep:

1. Animals will be fasted for 24 hrs prior to surgery.
2. Animals will be placed on the heating pad, anaesthetized and intubated. They will be kept hydrated by means of a Novalon ear vein catheter; IV saline (NaCl 9%) will be used at a dose of 300ml/hr. Inhaled 1.5%-2.5% Isoflurane will be used for continuous anesthesia intraoperatively (a vet tech will monitor the pigs).

Part I *of the the experiment will be used to monitor the forces exerted by intrathoracic pressure during coughing and breathing on the sternum. This is the only part where the animals will be alive and should last ~3hrs):*

1. **Between 3 and 7 force transducers will be attached to the midline of the sternum by standard midline sternotomy techniques; the transducers will be attached by bone screws and specially designed plates**. Disturbance of surrounding musculature will be minimized.
2. Pressure transducers will be placed on the bottom surface of the sternum and will be attached by means of bone cement if necessary.
3. Natural breathing forces will be measured first before placing the animal on a ventilator.
4. Coughing will be induced by two possible methods: either isolating the phrenic nerves in the neck and attaching them to an electric simulator while obstructing the airway or pressing the abdomen while obstructing the airway in the anaesthetized animals.

Part II *of the experiment will measure the forces at each rib joint in the euthanized pigs by simulating intrathoracic pressures.*

1. Animals from Part I will be euthanized (force transducers and other instrumentation will remain intact)
2. The dead animals will be ventilated to simulate intrathoracic pressures and the forces/ pressure will be measured.

Part III of *the experiment will measure the forces at each rib joint in the chemically fixed pigs (embalming fluid will be used and an experienced vet tech from Tufts University School of Veterinary Medicine will supervise the procedure)):*

1. Animals from Part II will be moved to necropsy in the Animal Medicine facility at UMMS.
2. Animals will be exsanguinated via catheterization of the common carotid; they will be flushed with heparin to minimize blood clotting and allow more complete perfusion/ fixation of the animals(10, 000 units per ml at a rate of 4ml/50-100lbs i.e. 20-30ml of 10,000 units of heparin/ml will be injected into the animal).
3. The heparnized blood will be flushed out via the external jugular vein by pumping Permaflow through the common carotid artery; when the outflow is almost clear the opening in the external jugular vein will be tied off and the embalming fluid will be pumped in at a flow rate of 3ml/minute until the animals are firm to touch.
4. Animals will be placed on a ventilator and the forces/pressures of breathing will be measured.
5. All animals will be disposed of at the end of the procedures according to the rules of the Department of Animal Medicine.

H2: Indicate how you will identify animals

Animals are tagged prior to purchase

I. ANESTHESIA, PRE-ANESTHETIC AGENTS (e.g., tranquilizers, narcotics), and ANESTHETIC AGENTS: Describe how these agents will be used in your studies. If none will be used, enter "none" in the "Agents" column.

I1 Frequency of administration	Species	Pre-anesthetic Agents & Anesthetic Agents	Dose	Route
Preop	Pigs	Telazol/Ketamine/Xylazine (standard prep: One vial of Telazol is reconstituted with 2.5ml ketamine (100 mg/ml) and 2.5 ml xylazine (100 mg/ml), gently mixed and then administered.	Telazol: 5mg/kg Ketamine: 2.5mg/kg Xylazine: 2.5mg/kg combined cocktail: 1ml/20kg	IM
Continuous		Isoflurane	1.5-2.5%	Inhalation

I2: MONITORING OF ANESTHESIA: In this space, describe (a) what will be monitored (e.g., corneal reflex, heart rate, respiration, response to noxious stimulus) and (b) how frequently each of these variables will be monitored.

Heart rate, oxygen saturation and temperature will be monitored continuously; jaw tension, corneal reflex, response to noxious stimulus, blood pressure **(indirectly)** and respiration will be monitored every 15-30 minutes **by a veterinary technician**.

J. SURVIVAL SURGERY

Complete this section if any animals will recover from anesthesia after a surgical procedure.
N/A

J1. In the space below, explain why it is necessary for the animals to recover from surgery.

J2. In the space below, describe pre-operative care (including physical examinations, lab tests, and any preconditioning apparatus). All anesthetic agents and pre-operative medications should be listed in Section I (above).

J3. Use the space below to describe in detail the surgical procedure(s) to be used.

J4. List all participating surgeons, technicians, and students, and indicate the number of years of experience with the particular species and procedures to be used.

NAME **YEARS OF EXPERIENCE**

J5. Describe immediate postoperative care, and provide dosage, route, and frequency of administration of specified analgesics for the first 48 hours.
Note that "**As needed**" or **" PRN**" do not constitute an acceptable schedule for analgesia**.**

Species	Analgesic Agents	Dose	Route	Frequency of Administration

J6. List the names of individual(s) who will check animals during recovery.

NAME **AREA CODE/TELEPHONE#**

J7. In the space below describe any expected or potential postoperative complications and describe how you will handle them.

J8. Do all your procedures comply with the GUIDELINES for Common Animal Procedures in the IACUC Instruction Book?	**Yes** **No**
J9. Where will surgery be performed?	**Rm**
J10. Where will animals be housed during recovery?	**Rm**
J11. Where will animals be housed after recovery?	**Rm**

J12. MULTIPLE SURVIVAL SURGERIES: If multiple survival surgeries will be performed on the same animal:

J12a: Justify the need for multiple surgeries. (See Instruction Booklet for valid reasons)

J12b: Give the species and number of animals that will have multiple survival surgeries.

J12c: Specify the time intervals between the surgical procedures.

K. ADMINISTRATION OF SUBSTANCES OTHER THAN ANESTHETICS

List all 1) Therapeutic and 2) Experimental/Study non-anesthetic agents that will be administered to the animals, including but not limited to: 1) drugs such as antibiotics, analgesics or local anesthetics used to minimize post-procedural pain, distress, or discomfort, and 2) drugs, infectious agents such as viruses or other substances under study. For drugs under study in the experimental component of your protocol, drug type or group (e.g., non-steroidal anti-inflammatory agents, α-adrenergic receptor blockers) will suffice; however specific drugs should be indicated if known.

1) Therapeutic agents N/A

Species	Agent	Dose	Route	Frequency & Total Duration

2) Experimental / Study Agents NONE				
Species	**Agent/Substance**	**Dose Range**	**Route**	**Frequency & Total Duration**

L. PROLONGED PHYSICAL RESTRAINT OR STRESS OF CONSCIOUS ANIMALS

Complete this section if any unanesthetized animals will be restrained, except when the restraint is for a brief examination, sample collection, or injection. Also complete if noxious stimuli will be administered, if food or water will be withheld, etc.

L1. Explain rationale for use of restraint or induction of stress:

NONE, all animals will be anaesthetized during experiment and will not be restrained otherwise. Animals will be however be fasted 24hrs before surgery.

L2. Describe device, dimensions, etc.:

N/A

L3. Duration and frequency animal will be confined to device:

N/A

L4. Observation intervals during confinement:

N/A

L5. Qualified faculty or staff making observations:
Name: **AREA CODE/TELEPHONE #:**

L6. Will pain or discomfort be induced? If yes, describe in detail using the space below.	**YES**	x	**NO**
L7. Will stimulation, including light and sound, be used to modify animal behavior? If yes, describe in detail.	**YES**	x	**NO**
L8. Will animals be fasted (food, approx. 24 hours and/or water, approx. 12 hours) or placed on a diet deficient in one or more nutrients? If yes, how long? How will the general well-being of the animal be determined? How often will the animal be weighed?	x **YES** 12-24 hrs Water only		**NO**
L9. Will analgesics, sedatives, or tranquilizers be used to provide additional restraint? If yes, make sure that the agent(s) are listed in Section I (above).	**YES**	x	**NO**

M. HAZARDOUS AGENT INFORMATION

M1. Will this project require the use of infectious biological agents? (pathogenic to man or animal) If Yes, an IBC form needs to be submitted	**YES**	X	**NO**
M2. Will this project require the use of recombinant DNA technology in live animals? If Yes, an IBC form needs to be submitted	**YES**	X	**NO**
M3. Will this project require the use of ionizing radiation in live animals? If Yes, radiation safety approval is required	**YES**	X	**NO**
M4. Will this project require the use of cytotoxic or chemotherapeutic chemicals in live animals? If Yes, an Environmental Health & Safety form needs to be submitted	**YES**	X	**NO**

M5. If you will be using any of the above agents, use this space to describe briefly what you will be doing.

However, you will need to get approval from the appropriate committee overseeing that activity. The IACUC will take no action on your application until approval has been obtained from the appropriate committee(s).
If M1.,M2., or M4. is checked "yes", please contact the IBC contact person from the EH&S office (508-856-3985) to obtain the proper forms and to begin the process of approval for Biosafety issues.
If M3 is checked "yes", please contact the Radiation Safety Office.

N. ADVERSE EFFECTS OF PROCEDURES AND EXPERIMENTS/ MONITORING AND MANAGEMENT

N1. What will be monitored to assess the presence of pain, discomfort, or other potential adverse effects caused by your studies? NOTE: This period includes the time from initiation of experiments until the animals are removed from the study; for surgically operated animals, this includes the time after anesthesia recovery (Section J) until animals are removed from the study. Check <u>all</u> that apply. N/A, no recovery from anesthesia is anticipated

Activity		**Appearance**		**Appetite**		**Behavior**	
Excreta		**Grooming**		**Guarding**		**Heart rate**	
Licking, biting		**Posture**		**Respiratory rate**		**Temperature**	
Vocalizing		**Weight loss**		**Wound site**		**Other**	
Laboratory tests or other evaluation							

N2. Indicate the frequency with which you will monitor your animals during and after all procedures. Please indicate both monitoring interval and total length of time.

Animals will be monitored during the first treatment phase but will be euthanized immediately after.

N3. Describe the conditions and complications that would lead to removal of an animal from the study and how this will be accomplished (e.g., stopping treatment and/or euthanasia).

O TERMINATION OF STUDY / EUTHANASIA

O1. Is death used as an endpoint in this study? Death as an endpoint means that the animal is permitted to die as a result of experimental manipulation, i.e. <u>exclusive</u> of planned euthanasia. If yes, explain why an earlier end point is not acceptable. (Studies using death as an endpoint are Category 3 (E) and require full IACUC review) **YES** X **NO**

O2. What criteria will be used to perform euthanasia earlier than planned?

O3. Other Use – Will animals be available for further use by other investigators?

O4. Describe the method(s) of euthanasia for each species or procedure. For injectable drugs, give name, dose and route. Must comply with 2000 Report of the American Veterinary Medical Association (AVMA) Panel on Euthanasia. See the IACUC Instruction Book. (Animal Welfare Act, 9 CFR, 2.31)(PHS Policy – Section B-2-3)(AVMA Panel on Euthanasia)

Species	**Method/Drug**	**Dose (mg/kg body wt.)**	**Route**
Pig	Fatal Plus **(sodium pentobarbital (390 mg/ml))**	1cc per 10lbs	IV

O5. Current rules require that after euthanasia, death be confirmed by using a second method. For example bilateral pneumothorax is done after euthanasia using CO2. Indicate below how you will double kill your animals.

Bilateral pneumothorax will be performed after an IV sodium pentobarbital (Fatal Plus ®) injection (1 mL/10 lbs).

P. APPLICANT'S CERTIFICATION

IACUC is charged with carrying out the rules and regulations of the Federal Government's Animal Welfare Act governing the care and use of animals in research and instruction. The Act stipulates that (a) Principal Investigators must give written assurance that the activities do not unnecessarily duplicate previous experiments; (b) procedures involving animals must avoid or minimize discomfort, distress, and pain to the animals; (c) Principal Investigators must consider alternatives to procedures that cause more than momentary or slight pain or distress to the animals and give a written description of methods used to determine that alternatives are not available; and (d) paralytic agents cannot be used in unanesthetized animals. Accordingly, the Applicant, who must be a member of the faculty holding Principal Investigator status, is required to read and sign the following certification:

BY SIGNING BELOW, I CERTIFY THE FOLLOWING:

1. **I am thoroughly familiar with the literature in the field of research proposed in this application, and I have determined that the research does not unnecessarily duplicate experiments, that appropriate non-animal models are not available, and that the research must be conducted on living animals.**

2. **I will abide by all UMMS policies and procedures regulating use of animals in instruction and research, by the provisions of the *PHS/NIH Guide for the Care and Use of Laboratory Animals*, and by all other applicable laws, policies, and regulations governing the use of animals in instruction and research.**

3. **I will supervise all experiments involving live animals. Furthermore, I will ensure that all listed participants are qualified or will be trained in proper procedures, including animal handling, anesthesia, surgery, post-procedural management, and euthanasia. Also, I will ensure that individuals not listed in the application will not have responsibility in experiments involving animals.**

4. **All listed personnel will read the IACUC-approved *Application to Use Vertebrate Animals in Research or Instruction* before undertaking any procedures on laboratory animals.**

5. **Survival surgery will be performed using standard aseptic procedures.**

6. **Animal Medicine clinical veterinary staff will be consulted as needed to ensure satisfactory veterinary care.**

7. **If I cannot be contacted, and animals in this project show evidence of illness or pain, emergency care, including euthanasia, may be administered at the discretion of the Animal Medicine veterinary staff.**

8. **This application meets all animal use and care requirements of the funding agencies that have been asked to support the research.**

9. **By signing below, I certify that <u>all</u> animal studies described in grant proposals using this protocol are described in this animal use application.**

Signature: _____ **Date:** _____

Appendix I: Mathcad program used to calculate in vitro stress distribution

Two-dimensional stress field due to multiple distributed loads

analytical solution from Malvern 1969

$$c := \begin{pmatrix} 1.2 \\ 3 \\ 5 \\ 7.7 \\ 10.2 \\ 12.5 \\ 15.3 \\ 16.7 \end{pmatrix} \quad d := \begin{pmatrix} 3 \\ 4.5 \\ 6.6 \\ 9.2 \\ 11.7 \\ 14.1 \\ 16.7 \\ 18.1 \end{pmatrix}$$

$ribs := 8$

$F := 360$

Force (N) in each rib is F/8

force per length, q, depends upon the width of each rib

$L := 19.5$

Length (cm)

$$q := \frac{\frac{F}{ribs}}{(d - c)}$$

Force per length (N/cm)

$$q = \begin{pmatrix} 25 \\ 30 \\ 28.125 \\ 30 \\ 30 \\ 28.125 \\ 32.143 \\ 32.143 \end{pmatrix}$$

$$\sigma x(x, y, i) := \frac{1}{\pi} \cdot \left[\int_{c_i}^{d_i} 2 q_i \cdot \frac{x^3}{\left[x^2 + (y - s)^2\right]^2} \, ds \right]$$

$i := 0..7$

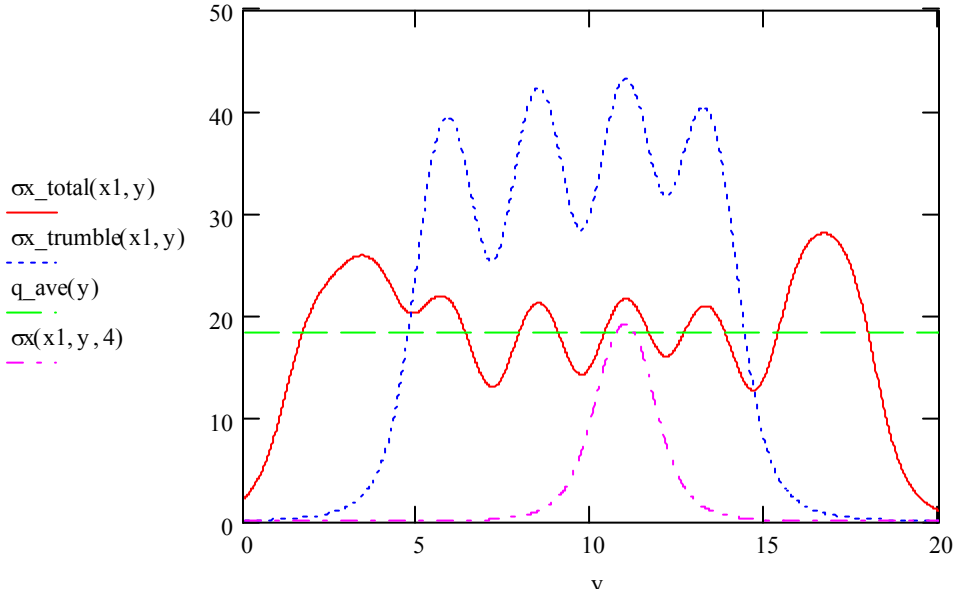
$$\sigma x\_total(x, y) := \left(\sum_i \sigma x(x, y, i) \right)$$

$j := 0..3$

$x1 := 1.2$

$$q\_ave(y) := \frac{F}{L}$$

$$\sigma x\_trumble(x, y) := \sum_j 2 \sigma x(x, y, 2 + j)$$



Data export, just right click on the data arrray and choose export

$k := 0..195$ distance along length in mm

$stress_{k,0} := \frac{k}{10}$ distance along length in cm

$stress_{k,1} := \sigma x\_total\left(x1, \frac{k}{10}\right)$ force per length along sternum at chosen x value

$stress_{k,2} := \sigma x\_trumble\left(x1, \frac{k}{10}\right)$

$stress_{k,3} := q\_ave\left(\frac{k}{10}\right)$

$stress_{0,4} := x1$

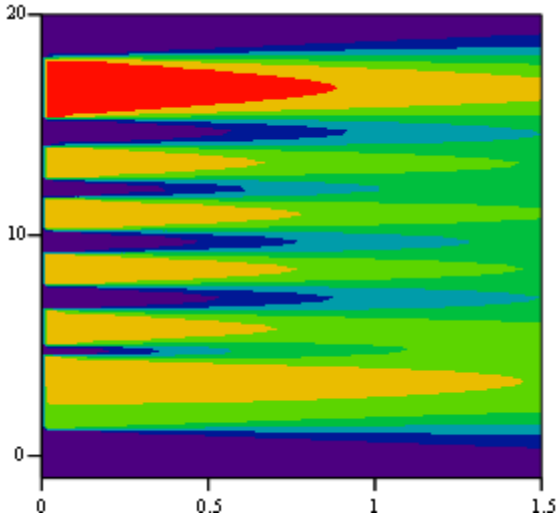
x position in cm

$stress =$

	0	1	2	3
0	0	2.275	0.114	18.462
1	0.1	2.638	0.122	18.462
2	0.2	3.066	0.13	18.462
3	0.3	3.57	0.138	18.462
4	0.4	4.161	0.148	18.462
5	0.5	4.851	0.158	18.462
6	0.6	5.648	0.169	18.462
7	0.7	6.559	0.181	18.462
8	0.8	7.584	0.194	18.462
9	0.9	8.717	0.209	18.462
10	1	9.943	0.225	18.462
11	1.1	11.238	0.243	18.462
12	1.2	12.571	0.262	18.462
13	1.3	13.906	0.284	18.462
14	1.4	15.208	0.308	18.462
15	1.5	16.448	0.334	18.462

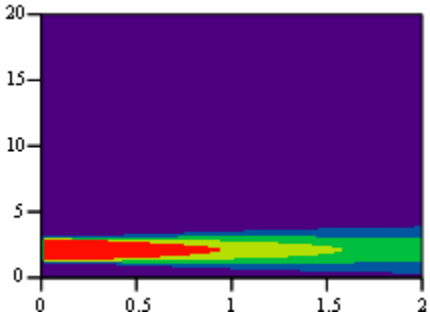
$i := 0..7$

$$\sigma x\_total(x,y) := \left(\sum_i \sigma x(x,y,i) \right)$$



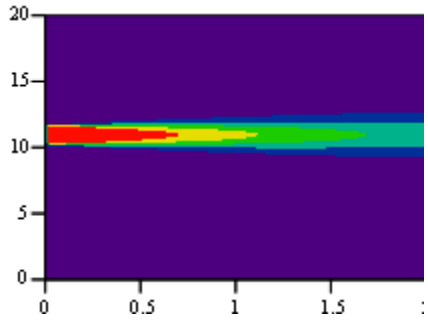
σx_total

$\sigma x0(x,y) := \sigma x(x,y,0)$



$\sigma x0$

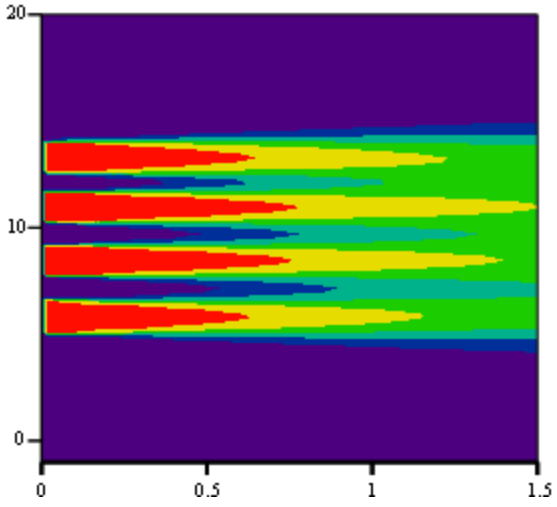
$\sigma x4(x,y) := \sigma x(x,y,4)$



$\sigma x4$

$j := 0..3$

$$\sigma x\_trumble(x,y) := \sum_j 2\,\sigma x(x,y,2+j)$$



$\sigma x\_trumble$

Appendix J: Protocol used to generate density plots for cadaver sterna

Photoshop

Images were edited in photoshop by removing sections of cancellous bone so that only the cortical shell was visible and the remaining background was black. Although there was no defined boundary indicating where cancellous bone ended or cortical bone started, any inconsistencies due to subjectivity were eliminated by using three levels of bone removal: conservative, moderate, and liberal.

Unedited bone section

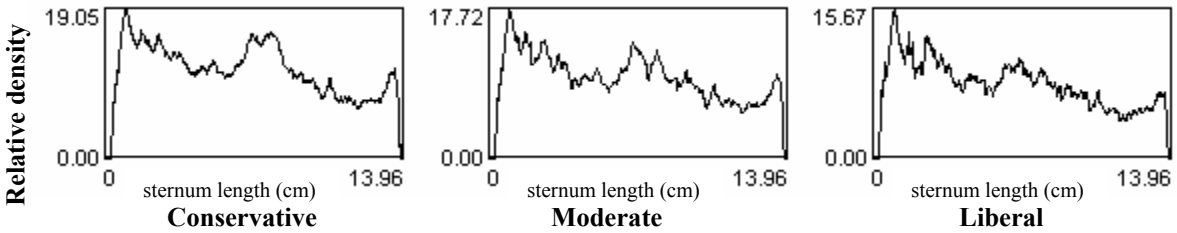
Manubrium Xiphoid

"Moderately" cropped bone sect

Scion

All three images (conservative, moderate, and liberal) for each cadaver sterna were analyzed for cortical bone density by plotting the relative number of pixels that were not black in each column of pixels. The directions for this process in Scion Image are as follows:

Open image: → file: import: (browse for image and select it): TIFF 8bit
View image: → options: scale to fit window
Scale image: → select invisible line tool from graphic toolbar & draw a line for a known distance.
→ analyze: set scale: (choose appropriate units & enter a known distance)
Area Plot: → options: profile plot options: (set details e.g. line plot; inverted)
→ select the boundary tool from graphic toolbar & enclose the image in the selection box
→ analyze: plot profile (click on the window with the plot)



→ file: export: (save as plot values, all file types under a new name to be opened later in Notepad and copied to MS Excel and used to find normalized averages for each cadaver sterna.)

Note that thresholded and non-thresholded values are the same so it is not necessary to use this function.

Appendix K: Plate component drawings in Pro-E