Effect of Storage Condition on Penetration Threshold of Porcine Skin Tissue

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EFFECT OF STORAGE CONDITION ON PENETRATION THRESHOLD OF PORCINE SKIN TISSUE

by

Jared Koser

A Thesis submitted to the Faculty of the Graduate School,
Marquette University,
in Partial Fulfillment of the Requirements for
the Degree of Master of Science

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ABSTRACT

EFFECT OF STORAGE CONDITION ON PENETRATION THRESHOLD OF PORCINE SKIN TISSUE

Jared Koser

Marquette University, 2023

Buried blast explosions create small projectiles which can become lodged in the tissue of personnel as far away as hundreds of meters. Without appropriate treatment, these lodged projectiles can become a source of infection and prolonged injury to soldiers in modern combat. Human cadavers can be used as surrogates for living humans for ballistic penetration testing, but human cadavers are frozen during transport and storage. The process of freezing and thawing the tissue before testing may change the biomechanical properties of the tissue. The goal of the current study was to investigate the effect of tissue storage on penetration threshold between fresh, refrigerated, and frozen tissue. Porcine tissues were used as a substitute for human cadaver since the skin is similar and allowed for hypothesis testing with more available tissues.

A custom-built pneumatic launcher was used to accelerate 3/16” stainless steel ball bearings toward porcine legs that were either tested fresh, following refrigerated storage, or following frozen storage. A generalized linear mixed model, accounting for within-animal dependence, owing to repeated observations, was found to be the most appropriate for these data and was used for analysis. The “generalized” model accommodated non-continuous observations, provided a straightforward way to implement the repeated measures, and provided a risk assessment for projectile penetration.

Both storage condition (p = 0.48) and leg (p = 0.07) were shown to be not significant and the 95% confidence intervals for those variables were overlapping. As all covariates were found to be non-significant, a single model containing all impacts was used to develop a $V_{50}$, or velocity at which 50% of impacts are expected to penetrate. From this model, 50% probability of penetration occurs at 137.3 m/s with 95% confidence intervals at 132.0 and 144.0 m/s.

In this study, the fresh legs and previously frozen legs allowed penetration at similar velocities indicating that previously frozen legs were acceptable surrogates for fresh legs. This study only compared the penetration threshold in tissues that had been stored in differing conditions. To truly study penetration, more conditions will need to be studied including the effect of projectile mass and material, the effect of projectile shape, and the effect of clothing or protective layers on penetration threshold.
ACKNOWLEDGEMENTS

Jared Koser

I would like to begin this work by thanking my wife, who has supported me throughout my unorthodox educational journey. It certainly does not get easier with age. Also, to my children, who have, for the last 2 years, given me reasons to put school and work aside and laugh and play for a while instead. I love all of you.

I would like to thank my advisor, Dr. Frank Pintar, for guiding this work. I am grateful for my committee members, Drs. Brian Stemper and Narayan Yoganandan, for taking the time to be a part of this process.

This work would not have been possible without statistical guidance from Drs. Anjishnu Banerjee and Sajal Chirvi, the testing and preparation assistance of Cassandra McCarthy, Joe LeSueur, and Austin Amato, or setup development assistance of Joe Frank and Elden Meyer. Nor would it have been possible without those who ensured this work was funded, including Dr. Michael Kleinberger, Dr. Carolyn Hampton, and Christy Stadig.
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LIST OF KEY TERMS

Perforation: A hole made by punching or boring through something.

Penetration: The action of making a way through or into something.

Penetration Threshold: (Unless specifically listed otherwise) The velocity at which 50% of impacts are expected to penetrate.
I. INTRODUCTION

Background

Buried blast explosions create small projectiles, including fragments of the explosive casing; sand, mud, and rocks; and metal debris, which can become lodged in the tissue of personnel as far away as hundreds of meters [1]. Without appropriate treatment, these lodged projectiles can become a source of infection and prolonged injury to soldiers in modern combat [2], [3]. The rate of combat casualties being killed in action before admission to a military treatment facility has decreased from 20.5% to 14.1% since the Vietnam War due to improvements in protective equipment and combat casualty care focused on blast survivability [4]. During that same time, however, the rate of casualties dying from wounds following treatment has risen from 3.3% to 5.3% as has the proportion of trauma-related infections [4], [5].

To protect soldiers from these risks, it is important to understand the conditions associated with skin perforation. Previous work in this field often relied on depth of penetration for comparative assessment [6], [7] and focused on the damage caused by the bullet as it passed through biological tissues [8], [9]. To study the risk of fragments lodging in the tissue, it is important to understand what projectile velocity is required to simply perforate the skin for a given projectile [10], [11]. This information can be used to develop appropriate protective systems to reduce the risk of penetration in areas not covered by hard armor including legs, arms, neck, and face.

Postmortem human subjects (PMHS) can be used as surrogates for living humans for ballistic penetration testing [12], [13], but human cadavers are frozen during transport and storage. A review of ballistic perforation studies by Breeze and Clasper found 9 instances where PMHS were used; of which, 4 used isolated skin samples, 4 used complete limbs, and 1 used both. Breeze’s evaluation of the data from these studies revealed that it was statistically more difficult to perforate the skin of a whole limb than isolated skin. Beyond this, conclusions were difficult for them to draw given the small sample sizes available and differences in, or complete omissions of, storage descriptions [14].
Porcine cadavers have also been used as human surrogates for penetration-type studies due to availability [7], [10]. The skin of the porcine animal has shown many similarities to that of human that make it a suitable substitute. Structurally, both skins are composed of three layers: the epidermis, dermis, and hypodermis. While the subdermal fat tends to be thicker in pigs, the epidermis thickness, dermal-epidermal thickness ratio, and basement membrane that connect the epidermis and dermis are similar in each [15]–[17].

Histologically, both skins have an epidermis composed of multiple layers of keratinocytes [16]. The dermal layer of each contains apocrine sweat glands, sebaceous glands, hair follicles, and interfollicular muscles. Additionally, the dermal collagen and elastin content of a pig is more similar to humans than other laboratory animals [18]. Pig and human skin also have similar compositions of lipids, proteins, and pigmentation, as well as blood vessels with similar size, orientation, and distribution [19].

Biomechanically, tensile stress/strain response of pig skin has shown to be a close match to that of human skin when accounting for collagen fiber orientation [20]. Porcine scalp demonstrated no difference in stretch at failure, however differences in elastic modulus and ultimate tensile strength were observed. It was recognized that the increased concentration of hair follicles in human scalp may affect the mechanical behavior of the tissue [21]. While significant Young’s modulus differences were observed in the individual layers, no difference was noticed in full-thickness skin between porcine and humans [22]–[24]. Additionally, initial slope of stress/strain curve, fracture strength, failure strain, and mean strain energy of porcine skin was found to be consistent with human skin [23], [24].

Problem

The process of freezing and thawing the tissue before testing may change the biomechanical properties of the tissue. The goal of the current study was to understand penetration threshold differences between fresh, refrigerated, and previously frozen tissue and to investigate factors that may contribute to these differences.
Present Status of the Problem

Previous studies have reported contradictory conclusions on the effect of storage conditions using porcine skin for mechanical testing. Some literature concludes that freezing does have a significant effect on tissue properties. In a review of mechanical properties comparing human and porcine skin before and after freezing, Ranamukhaarachchi et al. concluded that freezing decreased skin's stiffness and increased the energy required to break the stratum corneum using a microneedle [22]. Clavert et al. found that freezing significantly affected ultimate tensile failure and Young's modulus in human tendons and estimated this result to be linked to tissue dehydration during freezing [25]. Weeber et al. studied the compliance of porcine corneas before and after freezing and found that there was an 8% increase in elasticity and a 32% increase in the energy dissipated as heat under shear loading after freezing [26]. Chow and Zhang compared common storage methods, including refrigeration at 4°C for 48 hours and freezing at both -20°C and -80°C for up to 3 weeks, and the impact they have on the mechanical properties of bovine aortic tissues. They found that cold storage caused a reduction in the quantity of collagen fibers. They also found a higher stiffness after cold storage in the “stiff region” of the stress/strain curve; this region was described as that where the collagen fibers had been fully stretched [27].

Others concluded that freezing does not affect tissue properties. Linde and Sorensen examined the effect of storage condition on the elastic and viscoelastic properties of trabecular bone in compression. No significant changes in stiffness or elastic energy were observed after freezing for 100 days, nor after multiple freeze-thaw cycles [28]. A study on the biaxial tensile properties of frozen and fresh porcine arterial samples by Virues Delgadillo et al. indicated no significant differences in force-stretch curves [29]. O’Leary corroborated these results on porcine arterial tissue under tension, also finding no significant difference in elastic modulus after freezing for a variety of time lengths [30].

Still others noted that freezing affects only certain tissue properties or that it affects the properties of only certain tissues. Foutz et al. showed that while freezing at -70°C significantly
lowered failure strength of rat skin, it had no effect on Young’s modulus, loading response, or ultimate tensile strength [31]. Van Ee et al. focused on the response of cadaveric tissue to freezer storage and found that freeze-thaw cycles did not affect the stress-strain response of post-rigor muscles under uniaxial tension compared to never frozen muscle, but that muscle tissues frozen pre-rigor showed signs of stiffening immediately after thawing. Additionally, they found significant differences between fresh and frozen muscle’s failure stress and energy to failure but did not find a difference in strain at failure [32]. Stemper et al. pointed out a number of prior studies where freezing was found to affect the mechanics of a variety of soft tissues but found that freezing for up to 3 months did not significantly affect sub-failure stress, ultimate stress, or Young’s modulus of porcine aortas, highlighting the importance of testing each tissue instead of making assumptions based on other tissues [33].

Further others were inconclusive on the effect of storage condition. Maiden analyzed the use of cadaveric tissues and tissue-simulating gelatins for anatomical models to assess bullet wound trauma dynamics. He found consistent tensile strength in gelatins but was unable to draw tensile strength conclusions in cadaveric tissue due to the unpredictability of observed data [34]. Breeze and Clasper conducted a systematic review of skin perforation literature and predicted that cadavers that were frozen immediately after death would maintain their mechanical properties when thawed. They did not believe this assumption would hold for PMHS stored using preservatives. They supported this belief with conclusions of several papers but noted that these papers either contained a sample size that was too small to make statistical conclusions, failed to define key terms (including “fresh”, “penetration” or “perforation”, and “velocity for perforation”, which could be minimum threshold or 50% probability), failed to describe important experimental procedures (including tissue preparation), or failed to provide experimental data and were therefore insufficient to confirm or refute their hypothesis [14]. They also supported their conclusion by performing linear regression on each study with available data and performing a $\chi^2$ test to compare each regression line.
The two studies closest to the focus of the current study were conducted by Breeze et al. and James. The Breeze group studied the effects of refrigeration and freezing on depth of projectile penetration. Statistical analysis of impacts in their study was limited to 36 fresh, 16 refrigerated, and 10 frozen impacts which were then further divided by 3 projectile types. Because of the limited sample size, a complete statistical analysis was not possible. However, for those conditions with enough samples to perform a standard analysis of variance (ANOVA) test, no difference was found among each storage condition in the depth of penetration produced by impacts with a velocity less than 100 m/s [7]. In addition, a standard ANOVA, like that used in the Breeze study, does not allow for repeated measure analysis that would be appropriate when multiple impacts are taken to the same specimen. It was predicted that the factors driving depth of penetration differences would be similar to those responsible for penetration threshold.

Similar to this project, James conducted a study to determine the effect of storage conditions on skin perforation using porcine tissues. In his study, a total of 129 impacts were conducted on the legs from 3 animals. A probit model was used to estimate penetration probability under each storage condition (fresh, refrigerated, and frozen-thawed) for each projectile (0.49 g and 1.10 g). The 0.49 g projectile showed differences in penetration $V_{50}$ between the fresh and refrigerated legs; no frozen tissues were available for this projectile. No differences were observed in the penetration $V_{50}$ of the 1.10 g projectile across any of the 3 storage conditions [11]. However, the probit model of refrigerated tissues presented a different slope than the other two conditions. This study used matched-pair tests to control for inter-specimen variability in the fresh and refrigerated conditions, but no such control was included for the frozen-thawed specimen. Additionally, the statistical model chosen for this work failed to account for the repeated measures observed in testing.

While analyzing the literature, it became apparent that most studies only compared multiple populations, most using one-way ANOVA or a t-test, and didn’t estimate failure probability, which was a goal of the current study. Further, it was noticed that traditional failure
models used in biomechanics literature did not make appropriate statistical assumptions [35] and that new methods would need to be explored.

**Hypothesis**

Given the conflicting nature of current literature, the null hypothesis, that storage condition will not have a significant effect on penetration threshold, will be evaluated.
II. TEST APPARATUS

Launcher Background

Testing projectile penetration required a device that could be used to accelerate projectiles that simulate fragmentation towards tested tissues. Literature was consulted to determine what velocities are necessary to study fragmentation penetration injuries and mimic what is found in the combat environment. Sielicki detonated a person-borne IED similar to those worn in the November 2015 Paris attacks and dynamically tracked the individual fragmentation [36]. Fragments from the explosion had a mean initial velocity of 405 ± 110 m/s.

Champion examined the morbidity and mortality of mortars and artillery shells. They describe a 24 m radius from the blast site as death from either primary blast overpressure or high-velocity fragmentation; fragmentation velocities in this region can exceed 1000 m/s [1]. Beyond this, the radius for fragmentation injury can reach as far as 1500 m. Throughout this range, fragmentation velocity is shown to dissipate quickly due to the poor aerodynamics of the irregularly shaped pieces. All mortar fragments in Champion’s comparison had dropped below 300 m/s by 60 m, and even fragments from the largest artillery shell had dropped below 300 m/s by 150 m. The pattern of fragmentation velocity dissipating quickly is extended to small munitions as well. U.S. Army Training Circular 3-23.30 describes the most common fragmentation grenade, the M67, as having a maximum fragmentation range of 230 m but morbidity and casualty producing radii of only 5 and 15 m, respectively [37]. Most casualties that survive to reach surgical facilities will be struck by projectiles, with a mass between 0.2 and 0.5 g, traveling at velocities below 600 m/s [3].

Launcher Development and Characterization

The device used in this project was inspired by the gas gun portion of a split-Hopkinson (Kolsky) pressure bar device. In the split-Hopkinson pressure bar setup, a small reservoir of gas is filled to a set pressure determined through experimental analysis and monitored by an embedded pressure gauge. Solenoid valves control filling (from a source tank to the pressure reservoir), venting (to release pressure from the reservoir), and firing. When the firing valve is
opened, the pressure from the reservoir accelerates a small striker bar through a short barrel into
the incident bar creating a stress wave along the length of the incident bar that is used for
measuring the compressive stress-strain properties of materials being tested by the system [38].

The device used in this project also consisted of a gas source tank (helium), a pressure
reservoir, and a barrel (Figure 2.1). Pressure in this system’s reservoir was monitored by an
embedded pressure transducer and recorded using a digital data acquisition system (DTS, Seal
Beach, CA). Since one goal of this device was to be able to test penetration for a variety of
projectiles, the apparatus was designed to allow for interchangeable barrels. For this setup,
barrels were made of seamless stainless-steel tubing and tube fittings that would connect the
given size tubing to the solenoid valve.

![Figure 2.1: Test setup, including: (A) pellet catcher, (B) leg support device, (C) barrel and barrel support,
(D) firing valve and air reservoir, and (E) helium tank.](image)

The initial design included light gates to measure projectile velocity. This method proved
unreliable as some of the ball bearings were too small to cast a sufficient shadow to trigger the
gates. The next method considered was to add photosensors and LEDs to the barrel but was not
used for a number for reasons. The first was that a hole larger than the size of the ball bearing
may result in inconsistent launch speeds, but a hole smaller than the ball bearing would unlikely allow enough light to reach the sensor. The second was that the high pressures that were expected to be present in the barrel may be damaging to small LED and photosensor components. A third drawback to this solution was that the ball bearing builds velocity during the length of the barrel; by recording the velocity within the barrel, the recorded velocity may not match that of impact.

Instead, high-speed video (nac Image Technology, Simi Valley, CA), recorded at 40,000 samples per second, and video analysis software (Tracker, Aptos, CA) were used to track position of the ball bearing during its flight path; velocity was derived from this position data. This method was advantageous in that no equipment was at risk of damage and it allowed velocity measurements immediately prior to impact.

Acceleration of the projectile through the barrel requires that the force exerted on the projectile by the reservoir gases exceeds that of the combined air pressure in front of the projectile and frictional forces between the projectile and the walls of the barrel. As the length of the barrel grows longer, assuming the pressure behind the projectile continues to exceed resistance forces, the projectile has greater distance to accelerate before leaving the barrel. However, as the barrel length grows longer, maintaining greater pressure behind the projectile becomes increasingly difficult due to limited capacity of the pressure reservoir.

Using 0.177” BBs as a projectile (mass = 0.34 g), barrel lengths from three to six feet were tested to determine which length would allow a projectile to reach maximum velocity (Figure 2.2). In this system, the six-foot barrel resulted in the highest velocities with a peak velocity of nearly 460 m/s. This was ideal as the seamless steel tubing used for a barrel shipped in six-foot lengths and would require no cutting that may affect performance. For the sake of consistency, other diameter barrels used to test other projectile sizes were also kept at six-foot lengths.

Velocities achieved by this launching apparatus represent those of casualties whose injuries would unlikely be considered immediately life-threatening and who may see increased delay between injury and medical evaluation. The launching device designed for this project was
able to repeatably and reliably generate the velocities within the range of interest. Using a logarithmic relationship, projectile velocity was set by controlling launching pressure. Prior characterization of the launcher showed that error between actual velocity and expected velocity to be 0.002±7.268 m/s.

![Pressure-to-velocity curves developed by barrels of four different lengths.](image)

**Figure 2.2:** Pressure-to-velocity curves developed by barrels of four different lengths.

**Leg Support**

To support the leg during testing, a custom mount was developed that would hold the leg in position without restricting movement caused by impact (Figure 2.1, B). Legs were suspended from the support mount using string and screws placed in either the scapula or femoral head. An additional string, with one end tied around the ankle and the other placed under a metal plate, was used to rotate the leg such that the impact site was normal to the projectile path.
Safety Procedures and Considerations

All components of this setup are rated to withstand a minimum of 5000 psi despite testing not exceeding 2000 psi. This 2.5x safety factor is in addition to the manufacturer’s safety factor, i.e. the difference between the rated pressure and the actual burst strength.

To protect laboratory personnel from wayward or ricochet bearings, a bearing catch device was developed and placed down-range of the launcher (Figure 2.1, A). The device was made of 3/8” steel and was lined on the interior surfaces with foam insulation panels to further decrease projectile velocity and keep ball bearings inside the catch device. Plexiglass panels placed between the device and the controlling workstation provided additional protection.
III. TEST METHODS

Tissue Acquisition and Preparation

Due to scarcity and ethical considerations in acquiring fresh human cadaver legs, these tests were conducted on whole porcine cadaver legs stored under one of three storage conditions: fresh, refrigerated, or frozen. Porcine leg tissues were received as post-mortem, for which the institution does not require Institutional Animal Care and Use Committee (IACUC) approval. All test protocols, however, were approved by the local Safety Committee and by the Research and Development Committee. Multiple legs were taken from each animal, reducing the number of animals used.

Fresh tissues were obtained within 30 min of sacrifice and kept in an incubator at 39°C during transport to keep legs at in-vivo temperatures. All fresh tests were conducted within 2 hours of arrival at the laboratory. Refrigerated legs were received fresh, stored at 4°C and tested within 3 weeks. Before testing, legs were removed from the refrigerator and allowed to warm at room temperature overnight. Frozen legs were initially stored in a -40°C deep freezer for an average of 14 weeks before testing. Prior to testing, frozen legs were thawed in the walk-in refrigerator at 4°C for 3 days before being allowed to warm at room temperature overnight. Skin temperature was measured externally, at the impact site, prior to each test using a laser thermometer (Fisher Scientific, Waltham, MA).

Because of the limited availability of fresh porcine tissues during the two years of data collection, more legs were tested in the frozen condition than in either of the two others (Figure 3). An interim power analysis, using coefficient sizes and standard errors from preliminary data already available, was used to estimate potential effect sizes. The interim analysis revealed that there was more than 80% statistical power for the proposed experimental design, assuming an overall type I error level of 0.05 and additionally adjusting for potential correlation for within pig measurements. Additionally, due to the limited availability of tissue, both front and rear legs were used in this study, and legs were harvested from pigs ranging in size from 70-170 lbs. Each of
these variables, aspect (front/rear) and pig mass, were included in the statistical analysis to test their effects.

**Testing Procedures**

A 3/16" stainless steel ball-bearing (BC Precision, Chattanooga, TN), with a mass of 0.44 g, was selected to represent battlefield fragmentation for all impacts. Following the procedures described by the U.S. Army’s Ballistic Research Laboratory [39], as well as the U.S. Army Test and Evaluation Command [40], velocity for each shot was determined by the results of previous shots, where velocity was increased following non-penetrating impacts and decreased in the case of penetration. Penetration of the leg was defined as the projectile perforating the epidermal and dermal layers. Thus, projectiles that were lodged in the hypodermis were treated as a penetration. Penetration was confirmed using visual inspection of the impact site, high-speed video of the event, and post-test dissection of the leg. Dissection occurred upon completion of all shots to prevent tissue damage from affecting results. Initial impact velocity for each tissue storage condition was based on preliminary data not included in this study. Later into the study, some velocities were chosen to fill in missing data across the statistical region of interest.

To maximize the use of each leg, multiple shots were taken on each leg. Areas of the leg without immediately underlying bone were marked out. Then, impact locations were randomized within these regions. The wounding track of a smooth 25-caliber projectile, which is slightly larger than what was used in the current study, was found to not exceed 30 mm [41], [42]. So, to prevent potential wounding tracks from overlapping, shots were spaced at least 50 mm apart within the leg.

![Figure 3.1: Test matrix](image-url)
IV: STATISTICAL ANALYSIS

Descriptions of SAS Models

Historically, the lab has used a survival (or failure) analysis model to estimate injury (penetration, in this study) probability given the available data [43], [44]. This was done using SAS procedure LIFEREG. Since multiple shots were taken into the same specimen in these tests, statistical analysis to properly account for repeated measures was needed. Since LIFEREG assumes independence between observations and does not have a method to handle repeated measures, it was determined to not be an appropriate method for these data. Had each specimen only contained 1 shot, repeated measures analysis would not be necessary and LIFEREG would have been an appropriate choice.

Instead, a generalized linear mixed model (GENMOD), accounting for within-animal dependence, owing to repeated observations, was found to be the most appropriate for these data and was used for analysis. The “generalized” model accommodated non-continuous observations, in this case, binary observations (penetration versus non-penetration), provided a straight-forward way to implement the repeated measures, and provided a risk estimate for projectile penetration through the use of a logit link function. In this model, velocity was used as an independent variable for penetration, storage condition and leg (front or rear) as covariates, and the animal number as a repeated measure. Wherever appropriate, covariates were encoded as categorical (class) variables and analyzed by the use of appropriate dummy (indicator variables). Type 3 analysis tests were performed to determine the overall significance of each covariate, while standard Wald tests were used to provide information about the levels within the categorical variable. Results from this model will be covered in chapter 5.

As use of this method is uncommon in injury biomechanics literature, due to most studies having a reasonable assumption of independent samples, other commonly used statistical procedures from SAS Studio (v3.8) were explored for comparison. These included a semi-parametric survival analysis (PHREG), a linear mixed model (MIXED), and a non-linear mixed model (NLMIXED).
Each of the statistical analysis methods mentioned would give results based on user input (in this case, tissue storage condition would be found to not be significant in all of them), but each comes with its own set of assumptions. As each procedure is discussed over the subsequent paragraphs, sample code used to execute each procedure will be provided along with the procedure’s results displayed in both analytical tables and graphical figures.

Since this analysis was to determine at which point does the skin fail to prevent the projectile from penetrating the leg, it would be logical to assume that a traditional survival/failure analysis using either SAS’s provided PHREG or LIFEREG procedures would be the best choice. However, as previously mentioned, these methods assume independence between observations. In this study, multiple shots (repeated measures) were taken into each pig, and it can be expected that the results within each subject would be correlated. Therefore, statistical analysis of these tests must account for repeated measures. Like LIFEREG, PHREG does not have this function built into it, and while it may be possible to manually build this feature into a model, the process for doing so is something that can be easily error-prone for users without a strong statistical background.

```
proc genmod data=ARL2;
   class StorCond(ref="Fresh") PigNumber;
   model Penetrate(event='1') = ProjectileVelocity StorCond / dist=binomial link=logit type3;
   repeated subject=PigNumber / type=exch covb corrw;
   estimate "StorCond" StorCond 1 -1 / exp;
   output out=genmod pred=Predicted upper=UpperCL lower=LowerCL;
run;
```

*Figure 4.1: Sample code to execute the GENMOD procedure in SAS.*
proc lifereg data=ARL2;
class StorCond;
model ProjectileVelocity*Penetrates(\(\theta\)) = StorCond / dist=weibull;
output out=LIFEREG cdf=prob;
run;

Figure 4.2: Sample code to execute the LIFEREG procedure in SAS. Penetrations were considered observed events; non-penetrations were right censored. A Weibull distribution was found to best fit the data.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>Wald Chi-Square</th>
<th>Pr &gt; ChiSq</th>
</tr>
</thead>
<tbody>
<tr>
<td>StorCond</td>
<td>2</td>
<td>0.5746</td>
<td>0.7503</td>
</tr>
</tbody>
</table>

Figure 4.3: LIFEREG analytical results.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DF</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>95% Confidence Limits</th>
<th>Chi-Square</th>
<th>Pr &gt; ChiSq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>5.0692</td>
<td>0.0130</td>
<td>5.0438 5.0946</td>
<td>153010</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>StorCond Fresh</td>
<td>1</td>
<td>-0.0125</td>
<td>0.0197</td>
<td>-0.0511 0.0261</td>
<td>0.40</td>
<td>0.5250</td>
</tr>
<tr>
<td>StorCond Frozen</td>
<td>1</td>
<td>-0.0116</td>
<td>0.0172</td>
<td>-0.0454 0.0222</td>
<td>0.45</td>
<td>0.5013</td>
</tr>
<tr>
<td>StorCond Never Frozen</td>
<td>0</td>
<td>0.0000</td>
<td>.</td>
<td>. . .</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Scale</td>
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<td>0.0888</td>
<td>0.0048</td>
<td>0.0799 0.0986</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Weibull Shape</td>
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<td>11.2631</td>
<td>0.6038</td>
<td>10.1397 12.5110</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

Figure 4.4: LIFEREG graphical results.
proc phreg data=ARL2 plots(overlay)=survival;
class StorCond;
model ProjectileVelocity*Penetrate(θ) = StorCond;
baseline covariates=ARL2 out=PHREG survival=_all_/rowid=StorCond;
run;

Figure 4.5: Sample code to execute the PHREG procedure in SAS.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>Wald Chi-Square</th>
<th>Pr &gt; ChiSq</th>
</tr>
</thead>
<tbody>
<tr>
<td>StorCond</td>
<td>2</td>
<td>0.8482</td>
<td>0.6543</td>
</tr>
</tbody>
</table>

Type 3 Tests

Figure 4.6: PHREG analytical results.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DF</th>
<th>Parameter Estimate</th>
<th>Standard Error</th>
<th>Chi-Square</th>
<th>Pr &gt; ChiSq</th>
<th>Hazard Ratio</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>StorCond Fresh</td>
<td>1</td>
<td>0.10289</td>
<td>0.22254</td>
<td>0.2138</td>
<td>0.6438</td>
<td>1.108</td>
<td>StorCond Fresh</td>
</tr>
<tr>
<td>StorCond Frozen</td>
<td>1</td>
<td>0.18004</td>
<td>0.19550</td>
<td>0.8481</td>
<td>0.3571</td>
<td>1.197</td>
<td>StorCond Frozen</td>
</tr>
</tbody>
</table>

Analysis of Maximum Likelihood Estimates

Figure 4.7: PHREG graphical results. Note that this procedure models survival probability (which is the equivalent of "1 - penetration probability").
SAS’s MIXED procedure includes a repeated statement making it easy to account for repeated measures within a study, but the estimated probability function output from this procedure will be linear. Previous work has shown that linear models are often not the best fit for modeling response of biological tissues making the MIXED procedure a poor choice for this type of testing [45]. NLMIXED will allow a non-linear estimated probability function but remains a poor choice for this study for two reasons. The first is that the user needs to build their own model which may be unrealistic for those without statistical backgrounds. The second problem with this procedure is that the estimated output assumes a continuous observation where these data are described by a binary, penetrate/non-penetrate model.

```sas
class StorCond(ref="Fresh") PigNumber;
model Penetrate = ProjectileVelocity StorCond / s outp=MIXED;
repeated / type=vc sub=PigNumber;
run;
```

*Figure 4.8: Sample code to execute the MIXED procedure in SAS.*

| Effect      | Estimate | Std Error | DF  | t Value | Pr > |t|
|-------------|----------|-----------|-----|---------|------|
| Intercept   | 1.4226   | 0.2199    | 18  | -6.47   | <.0001|
| ProjectileVelocity | 0.01384 | 0.001554  | 263 | 8.77    | <.0001|
| StorCond    | 0.06101  | 0.06508   | 5   | 0.94    | 0.3916|
| Never Frozen| 0.04198  | 0.06850   | 5   | 0.61    | 0.5668|
| Fresh       | 0        |           |     |         |       |

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProjectileVelocity</td>
<td>1</td>
<td>263</td>
<td>76.95</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>StorCond</td>
<td>2</td>
<td>5</td>
<td>0.44</td>
<td>0.6642</td>
</tr>
</tbody>
</table>

*Figure 4.9: MIXED analytical results.*
Figure 4.10: MIXED graphical results.

```sas
proc nlmixed data=ARL2;
    bounds gamma > 0;
    lnp   = b0 - b1*(StorCond2-1) + z1;
    alpha = exp(-lnp);
    G_t   = exp(-(alpha*ProjectileVelocity)**gamma);
    g     = gamma*alpha*((alpha*ProjectileVelocity)**(gamma-1))*G_t;
    ll    = (Penetrate=1)*log(g) + (Penetrate=0)*log(G_t);
    model ProjectileVelocity ~ general(ll);
    random z1 ~ normal(0,0) subject=PigNumber;
    predict 1-G_t out=NL MIXED;
run;
```

Figure 4.11: Sample code to execute the NLMIXED procedure in SAS.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>DF</th>
<th>t Value</th>
<th>Pr &gt;</th>
<th>95% Confidence Limits</th>
<th>Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>gamma</td>
<td>11.2389</td>
<td>0.6031</td>
<td>18</td>
<td>18.63</td>
<td>&lt;.0001</td>
<td>9.9718</td>
<td>12.5060</td>
</tr>
<tr>
<td>b0</td>
<td>5.0622</td>
<td>0.01336</td>
<td>18</td>
<td>378.84</td>
<td>&lt;.0001</td>
<td>5.0341</td>
<td>5.0902</td>
</tr>
<tr>
<td>b1</td>
<td>0.000707</td>
<td>0.009494</td>
<td>18</td>
<td>0.07</td>
<td>0.9415</td>
<td>-0.01924</td>
<td>0.02065</td>
</tr>
</tbody>
</table>

Figure 4.12: NLMIXED analytical results.
GENMOD Curve Fitting and Confidence Intervals

One downside to using the GENMOD procedure, especially with smaller datasets, is the way probability is output. The model will only output a predicted probability, as well as upper and lower confidence limits, for each observation of the independent variable. For this project, that means a predicted probability of penetration for each impact velocity. If there are large gaps in the observations, a straight line will be used in figures to connect the points instead of continuing to fit along the model’s fit equation. Additionally, the procedure won’t use the equation that it generates to extrapolate up to a probability of 1 or down to a probability of 0. In this project, there are enough observations to make the curve appear filled, but it is possible to see the tails, especially for the fresh samples, not quite reach 0 and 1 (Figure 4.14).
To demonstrate how SAS-produced figures fit gaps in the observations, Figure 4.15 shows a probability curve generated for a separate project where the sample size is much smaller. In this figure, the linear region between 15-18 is an artifact of the way the model produces outputs.
To better reflect the results of the statistical model, the probability curve was reproduced in MATLAB, using Equations 4.1 and 4.2, so that estimates could be generated across the entire spectrum of observed values.

\[ Z = \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n + \text{Intercept}, \quad (4.1) \]

where the parameter estimates, \( \beta_n \), and intercept values were taken from the SAS parameter estimate table.

\[ \hat{Y} = \frac{e^Z}{1 + e^Z} \quad (4.2) \]

Figure 4.16 shows the SAS output of the smaller dataset in blue, which matches that shown above, and the MATLAB model in red. In this figure, the updated curve shape, in what was previously a gap in observed data and at the lower boundary, can easily be observed.

*Figure 4.16: GENMOD graphical results after MATLAB reconstruction from another project with a smaller sample size.*
Confidence intervals were derived by calculating standard error at each independent variable increment using the empirical covariance matrix, $[\text{CovMat}]$, provided by SAS and Equations 4.3 and 4.4.

$$SE_i = \sqrt{[C]'[\text{CovMat}][C]}, \quad \text{where} \ [C] = [1 \ X_1 \ X_2 \ \cdots \ X_n]$$  \hspace{1cm} (4.3)

$$CI = \frac{e^{\pm(1.96 \times SE)}}{1 + e^{\pm(1.96 \times SE)}}$$  \hspace{1cm} (4.4)

This subchapter used a dataset not related to this project to highlight a negative aspect of the GENMOD procedure more clearly. The corrective actions used in this subchapter to improve the graphical results were applied to the data from this project and will be covered in chapter 5.

**Machine Learning**

Machine learning was also evaluated as a predictive tool. In this method, a series of mathematical algorithms were trained on 90% of the collected data and tested against the remaining 10%. The model outputs included a cost function and an accuracy score; both are methods of determining how well the predicted results compare to the actual results. A loss function is the difference between the model’s predicted probability and the actual result for a single trial in the test population. The average of all these loss functions is called the cost function. The accuracy score is determined by rounding the predicted probability to an outcome of either 1 (penetrate) or 0 (non-penetrate) and determining at what percent does the predicted outcome match the actual outcome.

For this project, machine learning was done in Microsoft Excel (Microsoft Corporation, Redmond, WA) and was compared to logistic regression computed in the same workbook. The first step for both of these methods was to normalize all inputs such that they have a distribution where variance is equal to 1. An example is shown in Equation 4.5 using the velocity parameter.

$$\psi = \frac{V - \bar{V}}{\sigma_V}$$  \hspace{1cm} (4.5)
The resulting normalized inputs were then broken into training and test groups. A sample of this table can be found in Figure 4.17.

<table>
<thead>
<tr>
<th>Pig Mass</th>
<th>IsFront?</th>
<th>IsFresh?</th>
<th>IsFrozen?</th>
<th>Depth Temp</th>
<th>Skin Thickness</th>
<th>Velocity</th>
<th>Penetrate</th>
<th>90%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.47209087</td>
<td>0.0076122802</td>
<td>0.6161212456</td>
<td>0.010202108</td>
<td>0.176523255</td>
<td>1.560898592</td>
<td>0.705215082</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.47209087</td>
<td>1.067567802</td>
<td>-0.6161212456</td>
<td>-0.010202108</td>
<td>0.345500654</td>
<td>2.530906094</td>
<td>0.200466162</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.47209087</td>
<td>0.0167567802</td>
<td>0.6161212456</td>
<td>0.010202108</td>
<td>0.077802168</td>
<td>1.87253236</td>
<td>0.288672175</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.47209087</td>
<td>0.176523255</td>
<td>0.6161212456</td>
<td>0.010202108</td>
<td>0.0020928018</td>
<td>0.21764318</td>
<td>0.46508719</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.47209087</td>
<td>-0.0018545065</td>
<td>0.6161212456</td>
<td>0.010202108</td>
<td>0.0119658205</td>
<td>0.44166925</td>
<td>1.106058126</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.47209087</td>
<td>0.1213702395</td>
<td>-0.6161212456</td>
<td>-0.010202108</td>
<td>0.112869466</td>
<td>-1.904741331</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>-0.47209087</td>
<td>-0.0018545065</td>
<td>0.6161212456</td>
<td>0.010202108</td>
<td>0.121341598</td>
<td>0.345500654</td>
<td>0.200466162</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.47209087</td>
<td>0.1213702395</td>
<td>-0.6161212456</td>
<td>-0.010202108</td>
<td>0.112869466</td>
<td>0.111196005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.47209087</td>
<td>0.1213702395</td>
<td>-0.6161212456</td>
<td>-0.010202108</td>
<td>0.112869466</td>
<td>-0.205286082</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.47209087</td>
<td>0.1213702395</td>
<td>-0.6161212456</td>
<td>-0.010202108</td>
<td>0.112869466</td>
<td>0.111196005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.47209087</td>
<td>0.1213702395</td>
<td>-0.6161212456</td>
<td>-0.010202108</td>
<td>0.112869466</td>
<td>-0.205286082</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.47209087</td>
<td>0.1213702395</td>
<td>-0.6161212456</td>
<td>-0.010202108</td>
<td>0.112869466</td>
<td>0.111196005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.47209087</td>
<td>0.1213702395</td>
<td>-0.6161212456</td>
<td>-0.010202108</td>
<td>0.112869466</td>
<td>-0.205286082</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.17: Sample table of normalized input values.

To compute the linear regression, a predicted penetration probability was computed for each observation using Equations 4.6 and 4.7.

\[
Z = \theta_1 X_1 + \theta_2 X_2 + \cdots + \theta_n X_n + \theta_0
\]  
\[
\hat{Y} = \frac{1}{1 + e^{-Z}}
\]  

An accuracy score and a loss function value were determined for each observation. Accuracy scores were determined by rounding the estimated probability to either 1 or 0 and comparing with the observed penetration/non-penetration result. Loss values were computed using Equation 4.8. The mean of the loss function values for each of the train and test groups were found to determine each group’s cost function.

\[
\ell = -Y \log \hat{Y} - (1 - Y) \log (1 - \hat{Y})
\]
Finally, Microsoft Excel’s Solver add-in was used to tune the linear regression model. Solver was permitted to change the $\theta_n$ values in Equation 4.6 until the minimum value for the training group’s cost function was found. The linear regression model resulted in 69.8% accuracy with a test group cost function of 0.2664 (Figure 4.18).

![Figure 4.18: Results of logistic regression via Microsoft Excel.](image)

The process used for a feed-forward neural network was extremely similar to that of the linear regression, described above, except for the addition of one layer of nodes prior to finding the predicted penetration probability. This layer of nodes used 2 values for each $\theta_n$, resulting in 2 $Z_2$ values as seen in Equation 4.9.

\[
Z_{21} = \theta_{11}X_1 + \theta_{21}X_2 + \cdots + \theta_{n1}X_n \\
Z_{22} = \theta_{12}X_1 + \theta_{22}X_2 + \cdots + \theta_{n2}X_n
\] (4.9)
These $Z_2$ values were then used in the activation function. This model used a popular tanh function (Equation 4.10), chosen for its computational simplicity while modeling nonlinear boundaries.

\[
A_{21} = \left( \frac{2}{1 + e^{-2Z_{21}}} \right) - 1 \\
A_{22} = \left( \frac{2}{1 + e^{-2Z_{22}}} \right) - 1
\]  

(4.10)

An additional set of coefficients were included at this layer resulting in a modified version of Equation 4.6, shown below as Equation 4.11.

\[
Z = \omega_1 A_{21} + \omega_2 A_{22} + \omega_0
\]  

(4.11)

Computing predicted penetration probabilities, accuracy score, and loss/cost functions then was conducted as described above and used Equations 4.7 and 4.8. Again, Microsoft Excel’s Solver add-in was used to tune the feed-forward neural network. Solver was permitted to change the $\theta_n$ and $\omega_n$ values in Equations 4.9 and 4.11 until the minimum value for the training group’s cost function was found. The neural network resulted in 70.2% accuracy with a test group cost function of 0.2627 (Figure 4.19).

These results are somewhat conflicting; the neural network has a better accuracy score but a worse cost function. This is likely due to the neural network not fully reaching 0 or 1 at the respective extremes, leading to greater differences between predicted and actual values at the extremes. Rationally, this model poses an issue, where a ball bearing with velocity equal to 0 m/s should be expected to have 0% probability of penetration and a ball bearing with velocity equal to infinity should have a 100% probability of penetration. Observationally, both of the two models appear to have a 50% penetration probability at approximately 140 m/s, but the slope of the models differs such that the neural network appears to cross the 20% threshold between 130-135 m/s and the linear regression appears to cross near 125 m/s.
The quality of the machine learning model could be improved or impaired by changing any of the following: the parameters that were provided to the algorithms, the number of layers in the neural network, or the type of algorithm used in the nodes. Adding and removing parameters in the model would cause changes in the cost function and accuracy scores, which would indicate how much of an effect that parameter had on the model. The problem with this method was that it did not provide a method for determining if the parameter was statistically significant, which was needed for the current study. Model quality could be also improved or impaired by adding layers or changing the activation function of nodes, but without a solid understanding of when each activation function is appropriate, a novice user could easily overfit a model. In addition, each added layer makes the probability estimation more complex and therefore less user-friendly for researchers trying to quickly estimate probability given a set of inputs.
Comparison of Statistical Models

As previously mentioned, each of the described statistical models would produce a penetration probability curve. GENMOD was selected as the most appropriate for this application based on the validity of assumptions in each model. In this section, the results of each model are compared to demonstrate how much statistical model selection effects the resulting probability curve.

Figure 4.20 shows the predicted penetration probability of fresh tissue from each model that was discussed earlier in this chapter. The machine learning: logistic regression (green) and GENMOD (maroon) curves are reasonably similar due to both being based on a linear regression of the input parameters. Similarly, the NLMIXED (violet) curve closely matches LIFEREG (dark blue) because the NLMIXED model is designed after the LIFEREG’s mathematics. Table 4.1 shows a comparison of velocities at given penetration probabilities for each model. A “♦” in the table indicates a value outside the 95% confidence intervals of GENMOD, the model found to be most appropriate for this application.

A Kruskal-Wallis test of the 79 SAS or machine learning exported values for each test resulted in an H-value ($\chi^2$-score) of -1425. Using a look-up table, given 473 degrees of freedom, at the 0.05 significance level, the resulting p-value was <0.001 [46]. A post-hoc Dunn’s test with the Sidak Adjustment to determine between which groups a difference existed, showed significant difference (p<0.001) from GENMOD in the LIFEREG and NLMIXED procedures emphasizing the necessity to make appropriate statistical assumptions. No difference was seen in MIXED or either of the machine learning curves (Table 4.2). PHREG exports probability values differently than the other models resulting in a different number of points with different X-axis values. Thus, it would not be appropriate to include the PHREG exports into the Kruskal-Wallis or Dunn’s tests. While a Kruskal-Wallis test is designed to be used on independent data, it can be used to demonstrate where differences lie when other comparative statistics are not available.
Figure 4.20: Graphical comparison of statistical models using only fresh samples. *PHREG is shown as (1 – Survival) so that the morphology matches the other models.

Table 4.1: Velocities at chosen predicted penetration probabilities for fresh tissue by each method. ♦ indicates a value outside the 95% confidence intervals of GENMOD.

<table>
<thead>
<tr>
<th>Predicted Probability</th>
<th>GENMOD</th>
<th>LIFEREG</th>
<th>PHREG</th>
<th>MIXED</th>
<th>NLMIXED</th>
<th>MLOGREG</th>
<th>MLTANH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>115.9</td>
<td>128.6</td>
<td>132.2</td>
<td>111.7</td>
<td>129.5</td>
<td>120.0</td>
<td>100.9</td>
</tr>
<tr>
<td>0.25</td>
<td>127.1</td>
<td>140.6</td>
<td>139.8</td>
<td>122.7</td>
<td>141.5</td>
<td>129.6</td>
<td>132.4</td>
</tr>
<tr>
<td>0.50</td>
<td>138.3</td>
<td>152.0</td>
<td>148.0</td>
<td>141.0</td>
<td>153.0</td>
<td>137.6</td>
<td>137.7</td>
</tr>
<tr>
<td>0.75</td>
<td>149.4</td>
<td>161.5</td>
<td>160.1</td>
<td>159.3</td>
<td>162.6</td>
<td>146.6</td>
<td>144.5</td>
</tr>
<tr>
<td>0.90</td>
<td>160.6</td>
<td>169.5</td>
<td>171.1</td>
<td>170.3</td>
<td>170.3</td>
<td>156.4</td>
<td>181.1</td>
</tr>
</tbody>
</table>

Table 4.2: Kruskal-Wallis post-hoc Dunn’s test p-values when compared to GENMOD.

<table>
<thead>
<tr>
<th>p-value</th>
<th>GENMOD</th>
<th>LIFEREG</th>
<th>PHREG</th>
<th>MIXED</th>
<th>NLMIXED</th>
<th>MLOGREG</th>
<th>MLTANH</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-value</td>
<td>--</td>
<td>&lt; 0.001</td>
<td>N/A</td>
<td>0.767</td>
<td>&lt; 0.001</td>
<td>0.948</td>
<td>0.724</td>
</tr>
</tbody>
</table>
V. RESULTS

Of the 64 impacts into fresh tissue, 31 penetrated and 33 failed to penetrate. In the refrigerated condition, 73 total impacts resulted in 41 penetrations and 32 non-penetrations. And of the 115 frozen impacts, 61 were penetrating and 54 were non-penetrating.

GENMOD: Storage Condition and Aspect

To determine whether significant changes in the penetrating velocity threshold occurred because of specimen storage condition, a generalized linear mixed model with storage condition and aspect (front or rear) as covariates was evaluated. In this model, both storage condition ($p = 0.48$) and aspect ($p = 0.07$) were shown to be not significant (Figure 5.1) and the confidence intervals for those variables were overlapping (Figures 5.2-5.6).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>95% Confidence Limits</th>
<th>Z</th>
<th>Pr &gt;</th>
<th>Z</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-13.6942</td>
<td>2.4945</td>
<td>-18.5833</td>
<td>-8.8052</td>
<td>-5.49</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>ProjectileVelocity</td>
<td>0.1010</td>
<td>0.0175</td>
<td>0.0867</td>
<td>0.1353</td>
<td>5.77</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>StorCond</td>
<td>Frozen</td>
<td>0.1454</td>
<td>0.5372</td>
<td>-0.9076</td>
<td>1.1964</td>
<td>0.27</td>
<td>0.7867</td>
</tr>
<tr>
<td>StorCond</td>
<td>Never Frozen</td>
<td>0.6185</td>
<td>0.6935</td>
<td>-0.7407</td>
<td>1.9777</td>
<td>0.89</td>
<td>0.3724</td>
</tr>
<tr>
<td>StorCond</td>
<td>Fresh</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Leg</td>
<td>Front</td>
<td>-0.6509</td>
<td>0.3251</td>
<td>-1.2881</td>
<td>-0.0136</td>
<td>-2.00</td>
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</tr>
<tr>
<td>Leg</td>
<td>Rear</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

Score Statistics For Type 3 GEE Analysis

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Chi-Square</th>
<th>Pr &gt; ChiSq</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProjectileVelocity</td>
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<td>0.0007</td>
</tr>
<tr>
<td>StorCond</td>
<td>2</td>
<td>1.45</td>
<td>0.4844</td>
</tr>
<tr>
<td>Leg</td>
<td>1</td>
<td>3.26</td>
<td>0.0711</td>
</tr>
</tbody>
</table>

Figure 5.1: GENMOD analytical results with both aspect and storage condition included.
Figure 5.2: GENMOD graphical results comparing effect of aspect in fresh tissue.

Figure 5.3: GENMOD graphical results comparing effect of aspect in never frozen tissue.
Figure 5.4: GENMOD graphical results comparing effect of aspect in previously frozen tissue.

Figure 5.5: GENMOD graphical results comparing effect of storage condition in rear legs.
Figure 5.6: GENMOD graphical results comparing effect of storage condition in front legs.

**GENMOD: Only Storage Condition**

Since aspect did not prove to be significant, it was removed from the model and the model was run again using storage condition as the only explanatory variable. Again, storage condition was shown to be non-significant (Figures 5.7 and 5.8).
### Analysis Of GEE Parameter Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>95% Confidence Limits</th>
<th>Z</th>
<th>Pr &gt;</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-13.6063</td>
<td>2.2557</td>
<td>-18.0274 -9.1852</td>
<td>-6.03</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>ProjectileVelocity</td>
<td>0.0904</td>
<td>0.0155</td>
<td>0.0073 -0.1296</td>
<td>0.19</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>StorCond</td>
<td>Frozen</td>
<td>0.0593</td>
<td>0.4526 -0.8281</td>
<td>0.9468</td>
<td>0.13</td>
<td>0.3957</td>
</tr>
<tr>
<td>StorCond</td>
<td>Never Frozen</td>
<td>0.3082</td>
<td>0.6393 -0.9449</td>
<td>1.5613</td>
<td>0.48</td>
<td>0.6297</td>
</tr>
<tr>
<td>StorCond</td>
<td>Fresh</td>
<td>0.0000</td>
<td>0.0000 0.0000</td>
<td>0.0000</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

### Score Statistics For Type 3 GEE Analysis

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Chi-Square</th>
<th>Pr &gt; ChiSq</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProjectileVelocity</td>
<td>1</td>
<td>11.43</td>
<td>0.0007</td>
</tr>
<tr>
<td>StorCond</td>
<td>2</td>
<td>0.42</td>
<td>0.8106</td>
</tr>
</tbody>
</table>

*Figure 5.7: GENMOD analytical results with only storage condition.*

*Figure 5.8: GENMOD graphical results with only storage condition as an explanatory variable.*
**GENMOD: Final Model**

As all covariates were found to be non-significant, a single model containing all impacts was used to develop a $V_{50}$, or velocity at which 50% of impacts are expected to penetrate. From this model, 50% probability of penetration, regardless of storage condition, occurs at 137.3 m/s with 95% confidence intervals at 132.0 and 144.0 m/s (Figure 5.9). A list of velocities at chosen penetration probabilities can be found in Table 5.1.

<table>
<thead>
<tr>
<th>Predicted Probability</th>
<th>Velocity [m/s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>114.8</td>
</tr>
<tr>
<td>0.25</td>
<td>126.0</td>
</tr>
<tr>
<td>0.50</td>
<td>137.3</td>
</tr>
<tr>
<td>0.75</td>
<td>148.5</td>
</tr>
<tr>
<td>0.90</td>
<td>159.8</td>
</tr>
</tbody>
</table>

**Figure 4.9.** Final predicted penetration model for 3/16-inch stainless steel ball bearing for any storage condition.
VI: DISCUSSION AND CONCLUSIONS

Evaluation of Hypothesis

The null hypothesis, that storage condition will not have a significant effect on penetration threshold, was found to be correct. No significant differences in threshold were observed using an advanced statistical method that accounted for potential biological specimen differences in a repeated-measures type calculation.

Discussion of Results

Because other literature had different conclusions to the results seen in this study, it is important to consider why that may be the case. The current study is primarily concerned with porcine skin and immediately underlying muscle; results from studies using different tissue types may not be the most comparable. This is demonstrated when comparing the results of Weeber et al. and Linde and Sorensen. While Weeber et al. concluded that freezing increased compliance of human [eye] lenses, Linde and Sorensen found that freezing (including through five cycles of thawing and refreezing) did not significantly affect human trabecular bone stiffness [26], [28].

Tissues from different species may also produce different results. Stemper, O’Leary, and Virues Delgadillo all found no difference in porcine aortic mechanical properties after freezing [29], [30], [33], while Chow found changes in bovine aortas under similar storage conditions [27]. Similarly, Ranamukhaarachchi et al. showed that freezing decreased force required to insert a microneedle in porcine skin, but that it did not affect the force required in human skin [22]. Even in ballistic penetration literature, Breeze et al. found that porcine specimens had a slightly higher penetration \( V_{50} \) than their PMHS and goat counterparts, likely due to greater skin thickness [14].

Further, even with same tissues from the same species, findings regarding effects of freezing at specific loading rates or of specific mechanical properties should not be used to make assumptions about what would be seen at different rates or the response of different mechanical properties. Foutz et al. showed that freezing lowered fracture strength of rat skin but did not alter loading response, ultimate tensile strength, or Young’s modulus [31]. Santiago et al. found that
failure strain of bovine liver was reduced nearly 50% after freezing compared to fresh while failure stress was not statistically different [47]. Van Ee et al. found significant differences between fresh and frozen muscle’s failure stress and energy to failure but did not find a difference in strain at failure [32].

Several other papers hypothesized that mechanical differences seen between fresh and frozen or refrigerated tissues may be linked to changes in moisture content [25], [48]. However, Breeze et al. pointed out that ballistic properties of tissue are believed to be primarily determined by the density of the target material and that muscle density is not altered by moisture content [7].

The launcher designed for this project was highly sufficient in accelerating projectiles to the velocities relevant to penetration, repeatably. However, the peak velocity that this system can achieve is limited to ~460 m/s, even as pressure is increased. Should future studies have a need to exceed the 460 m/s limit of this device, modifications would be needed. Given the pressure reservoir and firing valve used in this system, a 6-foot barrel was found to produce the highest velocities. However, increasing the volume of the pressure reservoir and length of the barrel would be expected to result in higher maximum velocities. Additionally, the valve used in this setup had an orifice diameter of only 0.215” to allow for quick opening and high-pressure rating. As barrel volume increases, either through increased length or increased diameter to support larger projectiles, increasing exit velocity requires increasing flow through the firing valve. Since larger valve orifices come at the cost of slower operation and lower pressure ratings, an alternate design to increase flow rate would be to combine several valves in parallel.

A similar design was used in helium-powered fragment launchers by the Army Materials and Mechanics Research Center (AMMRC) in the early 1970’s and were capable of firing a .22 caliber fragment up to 290 and 300 m/s [49]. Those setups used a shorter barrel (28” and 36”) and a solenoid valve with a smaller orifice (0.156”), which likely explains the reduced maximum velocity compared to that of the present research.

In this work, survival/failure analysis was determined to be an inappropriate statistical method due to the within subject dependence stemming from multiple observations per subject.
These repeated measures required the use of a generalized linear model that could account for them. In biomechanics studies where observations can be assumed independent, use of survival/failure analysis has one major advantage not available to the generalized linear model: censoring. Had this study had independent observations, penetrating tests could have been treated either as observed events or as left-censored events, with the assumption that actual threshold velocity for each penetrating observation isn’t known, rather it is known that the threshold was somewhere below the measured velocity [50]. Similarly, non-penetrating impacts could be treated as right-censored events because that observation only was tested to the extent of the observed velocity, at which point, the skin was still intact and no information beyond the observed velocity is available to determine actual threshold velocity for that observation.

Since previously frozen tissues are easier to acquire than fresh tissues and do not require immediate testing, future studies and validation work will be justifiably conducted with previously frozen tissues because of this work. Additionally, since porcine skin is often used as a surrogate for human skin due to its biological similarities, it can be expected that these results would apply to previously frozen human cadaver legs being an appropriate surrogate for fresh human legs. No comparisons between the penetration thresholds of pig and human are made as a result of this study.

**Future Work**

When designing protective equipment, it is important to be able to demonstrate reduced probability of penetration. In this study, penetration threshold curves of naked flesh were created to be used as a comparative tool for future work. During development of the penetration threshold curves, it became apparent that traditional failure models did not make appropriate statistical assumptions and that new methods would need to be explored.

To protect soldiers from the risks associated with fragmentation penetration, it is important to understand the conditions associated with skin perforation and, more specifically, what projectile velocity is the threshold for penetration. This study only compared the penetration threshold in tissues that had been stored in differing conditions. To truly study penetration, more
conditions will need to be studied including the effects of projectile mass and material, the effects of projectile shape and size, and the effects of clothing or protective layers.
BIBLIOGRAPHY


