Reliability and Validity of a Novel Muscle Contusion Device

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Context: Many models have been employed to replicate skeletal muscle injury associated with trauma; however, most are restricted to 1 level of severity.

Objective: To create and validate an injury-producing device that could generate multiple levels of injury severity.

Design: Validation study.

Patients or Other Participants: Twenty-six male Wistar rats, 3 to 4 months old.

Intervention(s): A contusion device was developed and its ability to deliver consistent impacts was validated alone and in the presence of an experimental animal. A free-falling mass (267 g) was adjusted to the desired height (40, 50, 60, or 70 cm) and then dropped.

Main Outcome Measure(s): Peak load, peak displacement, impulse, energy, and velocity peak were measured. Injury severity was determined using magnetic resonance imaging.

Results: Outcome measures observed from the device alone were different by height ($F_{18,136} = 21.807, P < .001, 1 - \beta = 1.0$). Outcomes using the experimental animals were also dependent on height ($F_{14,102} = 68.679, P < .001, 1 - \beta = 1.0$). Linear regression analyses indicated that height accounted for 17% to 89% of the variance.

Conclusions: Mild to moderate and moderate to severe injuries can be replicated with this device, which will be useful in evaluating clinical treatments on acute muscle injury.

Key Words: blunt trauma, magnetic resonance imaging, skeletal muscle injuries

Key Points

- When the muscle contusion device was used alone and with experimental animals, outcome measures (peak load, peak displacement, impulse, energy, and velocity peak) depended on the height from which the mass was dropped.
- The muscle contusion device can induce mild to moderate and moderate to severe injuries.

Many models have been created to replicate skeletal muscle injury resulting from trauma. Muscle contusion injuries resulting from high-energy impacts that rapidly compress local tissues are common in both the athletic and general populations. Although many devices can mimic the initial impact of this type of injury, they produce only 1 level of severity. Contusions were first introduced as a crush injury with an in situ technique of squeezing the muscle between forceps. Skeletal muscle trauma and inflammation were generated; but this mechanism of injury is quite different from that occurring in contact sports; in addition, it is difficult to elicit a specific level of injury. Crisco et al. devised an injury device that could generate a reproducible focal injury via a drop-mass technique, in which a known mass slides through a plastic tube to an impactor, directly contacting 1 focal area on the animal, typically the gastrocnemius muscle (GTN). This technique can be performed in vivo, better replicating injuries typically seen by clinicians. This technique has been used to study acute inflammation and the influence of therapeutic modalities. Although this device has much merit, it is limited by its inability to produce injury at different severities. Therefore, the purpose of our study was to create and validate an injury device that could provide multiple severities of acute injury.

METHODS

Contusion Device

The 18 × 18-in$^2$ (45.72 × 45.72-cm$^2$) base of the contusion device was made of 0.5-in (1.27-cm) thick aluminum (Figure 1). The animal rested on an aluminum pedestal (6 × 14 × 0.125 in [15.24 × 35.56 × 0.32 cm]), with its leg securely held in position with a clamp. The height-adjusted retaining device used a solenoid to hold the drop mass until it was activated by a foot pedal (Figure 1A). A steel plunger (0.5 × 5 in [1.27 × 12.70 cm]) was nestled inside 2 ceramic bushings to decrease friction (Figure 1B), while a predetermined weight (267 g) free fell, guided by two 0.5-in (1.27-cm) ceramic bearings on two 0.5-in (1.27-cm) steel rods (40-in [101.60-cm] tall). A standard meter stick was secured to the side of the device as a guide for height adjustment.

Displacement of the plunger from the resting position was measured with a linear variable displacement transducer (model DSD800su5; Daytronic Corp, Dayton, OH). A load cell (model 9712B500; Kistler Instruments, Amherst, NY) was installed in the base to measure the force transmitted through the animal’s extremities. A custom-written LabVIEW program (National Instruments, Austin, TX) was used to collect the sensor data at 100 KHz (DAQpad 6070E; National Instruments). Data analysis
was conducted using another custom-written LabVIEW program (National Instruments).

For each trial, the following variables were calculated. Peak displacement (mm) and peak velocity (mm/s) were determined from the displacement-time curve. Peak load (N) and applied impulse (kg-m/s) were determined from the load-time curve. Applied energy (J) was determined from the load-displacement curve. The applied impulse and applied energy were defined as the area under the corresponding curves during the loading phase only. The loading phase started when the load was greater than the threshold of 5 N and ended at the peak load.

Procedures

The first set of experiments only examined the performance of the device. Fifteen trials were conducted at drop heights of 40, 50, 60, and 70 cm. Measurements taken included peak displacement, peak velocity, peak load, impulse, and energy.

The second set of experiments included animal test subjects: 20 caged, sedentary, male Wistar rats, 3 to 4 months old. All experimental procedures were approved by The University Laboratory Animal Care and Use Committee. Animals were housed 2 per cage, were fed commercial rat chow food and tap water ad libitum, and were on a 12-hour light-dark cycle. Before injury, animals were euthanized via carbon dioxide inhalation and weighed; the hind limbs were shaved and cleaned with alcohol. The midbelly region of the GTN was determined through palpation and marked with a permanent marker. Each animal was positioned prone in the injury device with the hind limb fully extended inside the leg holder and clamped into position. This ensured an impact directly over the midbelly region of the GTN muscle. The plunger rested on the animal’s limb, and the animal’s body was secured with hook-and-loop tape to the pedestal. After the impact, the leg was removed from the device; the contralateral leg was secured in the holder, and the process was repeated.

Another set of animals (n = 6) had 1 hind limb injured (the contralateral leg was the uninjured control) and then scanned with magnetic resonance imaging (MRI) to evaluate the level of injury induced by the different drop heights (40, 50, 60, or 70 cm). The same procedures were used as in the prior animal experiment except that the animals inhaled an anesthetic (isoflurane: 5% per 1000 mL of oxygen initially, then 3% per 500 mL of oxygen as needed). After injury, MRI imaging was conducted on a 7-T horizontal system (Varian Medical Systems, Inc, Palo Alto, CA) with a quadrature birdcage resonator at 10 minutes and 6 hours after injury to evaluate the volume changes in the injured leg. A spin-echo sequence with TE = 11 milliseconds, 56 slices, and spatial resolution of 215 × 194 × 500 mm³ was used. In addition, at 6 hours after injury, quantitative T2 maps, measured using 5 TE values (11, 20, 30, 40, and 60 milliseconds) were performed. Using AMIRA (version 4.1.0; Visage Imaging, Inc, Carlsbad, CA), segmentation of 30 adjacent slices, starting at the top of the knee joint for each animal, was used to calculate the 3-dimensional volume of the hind limb, as well as the 3-dimensional T2 map (Figures 2 and 3). To estimate the effects of injury, the mean and SD of the T2 value of the control leg were calculated, and the number of pixels with a T2 value larger than the mean by 2 SDs or more was calculated and converted into an absolute volume.

Statistical Analysis

We conducted analysis of variance to examine the effect of height on all dependent variables. Regression analysis was also performed to determine the amount of variance that could be explained by the different heights. Statistical significance was established a priori at P < .05.

RESULTS

Outcomes measured from the device alone depended on drop height (F₁₈,₁₃₆ = 21.807, P < .001, 1 – β = 1.0). The outcomes measured using the experimental animal also depended on drop height (F₁₄,₁₀₂ = 68.679, P < .001, 1 – β = 1.0; Table 1). The T2 maps differed based on height (P = .013, 1 – β = .815; Figure 1); injury sustained from a 40-cm drop was different from that at 60 cm (P = .021). Limb volume was also different based on height (P = .040, 1 – β = .668; Figure 2); injury sustained from a 40-cm drop was different from that at 60 cm (P = .017). Linear regression analyses indicated that height accounted for 17% to 89% of the variance, depending on the variable of interest (Table 2).
DISCUSSION

Reliability and Influence of Height

We have constructed a device that can reproducibly generate different peak loads depending on drop height. In all cases, the dropped weight was constant, and the only variable changed throughout the experiment was height. Regression analysis showed that height influenced the type of injury produced (Table 2). Previous authors\(^2\),\(^3\),\(^7\) have tested drop masses at various heights but have not determined the influence of height on the variables of interest (eg, peak load, impulse). In our case, height explained 89% of the variance seen with the load generated by the device and about 66% of the load applied to the animal. Thus, more than half of the injuries generated by a given load can be attributed to the height or distance at which the drop begins to the actual impact.

Magnetic Resonance Imaging

Previous researchers\(^2\) have based the severity of injury on the size of the hematoma and other histologic methods. In addition, biomechanical assessments (eg, tissue failure,\(^2\) stress/strain\(^7\)) have also been used to validate devices. Although these markers are critical in assessing tissue function, many are quantified after tissue extraction. Using MRI, we have examined the level of injury severity induced by a drop mass with a live animal and intact muscles. Such imaging has been used repeatedly to determine the level and location of injuries.\(^12\)\(^-\)\(^14\) This clinical measurement provides a noninvasive method to examine tissue response and the time course of healing. Our MRI findings confirm that a contusion injury was induced (Figure 2). Despite our small sample (n = 6), we were able to demonstrate a difference based on height. Further analysis revealed that 2 levels of injury occurred with this device. In addition to traditional T2 maps, we also used MRI to calculate limb volume (Figure 3). Limb volumes have been used clinically as a method of determining the amount of inflammation or

Table 1. Outcomes as a Function of Height (Mean ± SD [Coefficient of Variation %])

<table>
<thead>
<tr>
<th>Height, cm</th>
<th>Peak Load, N(^a)</th>
<th>Peak Displacement, mm(^b)</th>
<th>Impulse, kg-m/s(^a)</th>
<th>Energy, J(^b)</th>
<th>Velocity, mm/s(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>718.9 ± 59.5 (8.3%)</td>
<td>1.52 ± 0.05 (3.3%)</td>
<td>0.34 ± 0.01 (3.3%)</td>
<td>0.112 ± 0.01 (6.6%)</td>
<td>1171 ± 50.0 (4.3%)</td>
</tr>
<tr>
<td>50</td>
<td>860.9 ± 12.8 (1.5%)</td>
<td>1.75 ± 0.06 (3.4%)</td>
<td>0.38 ± 0.01 (2.1%)</td>
<td>0.123 ± 0.01 (7.5%)</td>
<td>1298 ± 38.1 (2.9%)</td>
</tr>
<tr>
<td>60</td>
<td>935.8 ± 26.5 (2.8%)</td>
<td>1.81 ± 0.06 (3.3%)</td>
<td>0.40 ± 0.01 (1.3%)</td>
<td>0.137 ± 0.01 (7.2%)</td>
<td>1338 ± 42.6 (3.2%)</td>
</tr>
<tr>
<td>70</td>
<td>1032.7 ± 13.3 (1.3%)</td>
<td>1.72 ± 0.04 (2.3%)</td>
<td>0.42 ± 0.01 (2.8%)</td>
<td>0.141 ± 0.02 (10.8%)</td>
<td>1364 ± 19.0 (1.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Height, cm(^c)</th>
<th>Peak Load, N(^a)</th>
<th>Peak Displacement, mm(^d)</th>
<th>Impulse, kg-m/s(^a)</th>
<th>Energy, J(^d)</th>
<th>Velocity, mm/s(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>181.9 ± 14.5 (8.0%)</td>
<td>5.00 ± 0.24 (4.8%)</td>
<td>0.19 ± 0.02 (8.9%)</td>
<td>0.204 ± 0.03 (13.8%)</td>
<td>2401 ± 57.9 (2.4%)</td>
</tr>
<tr>
<td>50</td>
<td>213.6 ± 22.5 (10.5%)</td>
<td>5.08 ± 0.27 (5.3%)</td>
<td>0.21 ± 0.02 (8.8%)</td>
<td>0.215 ± 0.03 (13.7%)</td>
<td>2574 ± 58.2 (2.3%)</td>
</tr>
<tr>
<td>60</td>
<td>244.5 ± 18.6 (7.6%)</td>
<td>5.52 ± 0.22 (4.0%)</td>
<td>0.23 ± 0.01 (6.2%)</td>
<td>0.259 ± 0.03 (13.1%)</td>
<td>2816 ± 59.0 (2.1%)</td>
</tr>
</tbody>
</table>

\(^a\) All values different from each other (P < .001).

\(^b\) All values different except for 60 cm and 70 cm.

\(^c\) Because of the high number of fractures at the 70-cm height, the sample size was insufficient, and these data are not provided.

\(^d\) All values different except for 40 cm and 50 cm.
injury of a particular limb or joint. Based on the data collected from the T2 maps and calculated limb volumes, weight dropped from a height between 40 and 50 cm produced one level of injury (mild to moderate), which could be reproduced. When the drop height was increased to 60 cm or higher, another level of severity (moderate to severe) injury was sustained. Heights of 70 cm or greater are likely to result in fractures of the tibia and fibula.

**CLINICAL APPLICATIONS**

To advance clinical practice, clinical efficacy for treatment protocols must be demonstrated. Often, settings for treatment modalities are based on research performed with healthy participants, a situation that is not optimal. To better address clinical outcomes and therapeutic variables, we need to discover how the modality affects injured or patient populations. Our device offers a way to study treatment interventions and determine their efficacy based on injury severity. Determining physiologic changes associated with clinical modalities provides necessary evidence to move forward with outcomes-based measurements and clinical trials. Good animal and injury models supply the foundation for examining variables before patient populations are studied. Our future research will include examining the time course of the injuries through the use of MRI and determining the influence of clinical modalities on healing time.

The purpose of our study was to construct a new contusion device that would allow different degrees of injury severity to be generated. After construction, testing was performed with the device alone and with an animal model. We were then able to conclude that different degrees of injury can be induced with this device. The small sample that underwent MRI allowed us to confirm that we have developed a device that can generate mild to moderate and moderate to severe levels of injury.

**Table 2. Variance Explained by Height of Drop ($R^2$ Values)**

<table>
<thead>
<tr>
<th></th>
<th>Device Alone</th>
<th>Injured Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak load</td>
<td>0.894</td>
<td>0.659</td>
</tr>
<tr>
<td>Peak displacement</td>
<td>0.687</td>
<td>0.415</td>
</tr>
<tr>
<td>Impulse</td>
<td>0.851</td>
<td>0.510</td>
</tr>
<tr>
<td>Energy</td>
<td>0.527</td>
<td>0.347</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.661</td>
<td>0.891</td>
</tr>
<tr>
<td>T2</td>
<td>N/A</td>
<td>0.404</td>
</tr>
<tr>
<td>Limb volume</td>
<td>N/A</td>
<td>0.279</td>
</tr>
</tbody>
</table>

Abbreviation: N/A indicates not applicable.

a $P < .001$.

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**REFERENCES**


Nicole M. McBrier, PhD, ATC, and Thomas Neuberger, PhD, contributed to conception and design; acquisition and analysis and interpretation of the data; and drafting, critical revision, and final approval of the article. Nori Okita, MS, contributed to conception and design; acquisition and analysis and interpretation of the data; and drafting, critical revision, and final approval of the article. Andrew Webb, PhD, contributed to conception and design; acquisition and analysis and interpretation of the data; and drafting, critical revision, and final approval of the article. Neil Sharkey, PhD, contributed to conception and design, analysis and interpretation of the data, and critical revision and final approval of the article.

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