DO MULTIPLE FREEZE-THAW CYCLES AFFECT THE MULTIDIRECTIONAL INTERVERTEBRAL MOTION PARAMETERS OF THE PORCINE LUMBAR SPINE?

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INTRODUCTION:
In vitro experimental studies of the spine that examine mechanical properties or the effect of fixation devices are often performed on fresh, frozen cadaver material. Investigators often assume that the mechanical properties of these spines accurately reflect the in vivo conditions. Although the mechanical properties of spine specimens have been reported to not be affected by long term storage in a deep freezer[1] frozen storage has been shown to increase disc hydration (resulting in swelling) and alter the viscoelastic response to compression of the porcine disc.[2] Since many study designs require staged specimen preparation and biomechanical testing, the constancy of spinal motion behavior after multiple freeze-thaw cycles should be understood. However, we found no published studies describing the effect of multiple freeze-thaw cycles on the mechanical behavior of motion segments. The objective of this study was to determine the effect of multiple freeze-thaw cycles on the biomechanical parameters measured during dynamic pure moment loading in the porcine spine.

METHODS:
Ten lumbar functional spinal units were obtained from 4 female pigs with an average weight of 61.3 kg (range 57 to 69 kg). Specimens were harvested within 1 hour after death. After screening with x-ray to ensure there were no skeletal anomalies, surrounding non-ligamentous tissues were removed leaving the intact bony and ligamentous structures. Single motion segments were then potted in polymethylmethacrylate in circular acrylic fixtures such that the upper and lower fixtures were parallel. Specimens were tested in a custom dynamic spine testing apparatus with a 3-axis gimbal and stepper motors allowing adjustment of loading rates and limits. Forces and moments were recorded by a 6 component load cell and angular displacement was measured by miniature tilt and rotation sensors. Motion was induced in flexion-extension, lateral bending, and axial rotation with continuous cycles under pure moment loading up to ±5 Nm at the rate of 3 degrees/sec. Each load was applied 5 times with the data from the 5th cycle used for analysis. Baseline data (BL) was collected within 3 hours after sacrifice. Specimens were then wrapped in towels dampened with normal saline and frozen (-20 degrees C.) in a thick plastic bag. Motion segments were then thawed at room temperature (21 degrees C.) for 12 hours prior to repeat testing and refreezing. Before each post-thaw testing axial compression of 300 N was applied for 30 minutes to expel excess fluid and avoid disc hydration beyond the normal in vivo range. Specimens were kept moist with 0.9% saline soaked towels during all testing. They were thawed, tested and refrozen every 48 to 72 hours for a total of 88% in lateral bending (p = 0.005) after the initial freeze-thaw cycle but no subsequent differences were found among the post-thaw measures (Figure 2). No significant difference was found in the size of TZ during axial rotation among all conditions.

The TZ slope (stiffness) increased 26 % in flexion-extension (p=0.0007) and 22 % in lateral bending (p=0.0001) after the initial freeze-thaw cycle (Figure 3). However, no significant differences were found among the post-thaw tests. No significant difference in TZ slope was found during axial rotation.

RESULTS:
There was no significant change in the size or slope of the EZ or the width of the HZ at any time point for any of the testing conditions (flexion-extension, lateral bending, and axial rotation).

The TZ size was reduced to 83 % in flexion-extension (p = 0.005) and 88% in lateral bending (p = 0.005) after the initial freeze-thaw cycle but no subsequent differences were found among the post-thaw measures (Figure 2). No significant difference was found in the size of TZ during axial rotation among all conditions.

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DISCUSSION:
We measured the effect of multiple freeze-thaw cycles on dynamic motion parameters in porcine lumbar motion segments and found that TZ size and TZ slope (neutral zone stiffness) changed significantly after initial freezing but were not altered by subsequent freeze-thaw cycles. There was no significant change in hysteresis as a result of freezing. Our results suggest that the mechanical behaviors measured after initial freezing were reasonably stable in these specimens. This suggests that porcine spine specimens which have been frozen and thawed multiple times can be used in biomechanical testing.

Previous studies using human spines found that biomechanical properties measured during static loading were not affected by freezing and thawing. Our results are not inconsistent with these reports. We saw no change in the EZ or HZ parameters which reflect overall specimen stiffness. However, we found the TZ size and slope (reflecting mechanical behavior in the neutral zone) to be significantly affected by the initial freeze-thaw cycle in these porcine spines. Since water content is an important factor in determining deformation characteristics of the disc, it is possible that the conformational changes brought about by freezing alter the mechanical properties of the nucleus or inner annulus. The response of porcine and human discs to freezing may be different due to higher water content in the young porcine nucleus, which might cause more ice crystal formation and nuclear expansion with freezing.

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REFERENCES

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