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Effect of Multiple Freeze-Thaw Cycles on Intervertebral Dynamic Motion Characteristics in the Porcine Lumbar Spine

Michio Hongo, M.D.¹, Ralph E. Gay, M.D., DC^{1,2}, Jui-Ting Hsu, Ph.D.¹, Kristin D. Zhao, M.S.¹, Brice Ilharreborde, M.D.³, Lawrence J. Berglund, B.S.¹, and Kai-Nan An, Ph.D.¹

¹*Biomechanics Laboratory, Division of Orthopedic Research, Mayo Clinic College of Medicine, Rochester, MN USA*

²*Department of Physical Medicine and Rehabilitation, Mayo Clinic College of Medicine, Rochester, MN USA*

Abstract

Fresh frozen spine specimens are commonly used in biomechanical investigations of the spine. Since many study designs require staged preparation and testing, the effect of multiple freeze-thaw cycles on motion behavior should be understood. The objective of this study was to investigate the effect of multiple freeze-thaw cycles on the biomechanical parameters measured during dynamic pure moment loading. Ten porcine lumbar motion segments were harvested immediately after death and potted in acrylic fixtures. Specimens were tested in continuous pure moment flexion-extension, lateral flexion, and rotation cycles up to a limit of ± 5 Nm. Moment-angular displacement data were analyzed and parameters quantified including range of motion, elastic zone, transitional zone (neutral region) size and slope, and width of the hysteresis loop. All specimens were tested at baseline and after each of 3 subsequent cycles of freezing and thawing. The transitional zone size decreased and the transitional zone slope increased during flexion-extension and lateral bending after the initial freeze-thaw cycle. These parameters were not altered after subsequent cycles. No significant change was observed in the elastic zone or width of hysteresis loop. Although freezing porcine spine specimens increased the stiffness in the neutral region of motion, up to three subsequent cycles of freezing and thawing did not further affect these motion characteristics. This suggests that data obtained from porcine spines which have been frozen and thawed multiple times are stable after initial freezing.

Keywords

lumbar spine; biomechanics; freeze; intervertebral disk; porcine; specimen handling

1. Introduction

It is important to understand intervertebral motion kinematics when studying conditions of the spine or developing spinal devices. Studies addressing these topics are often performed on

Corresponding Author: Ralph E. Gay, M.D., DC, Department of Physical Medicine and Rehabilitation, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905, USA, Phone: 507-266-8913, Fax: 507-266-1561, E-mail: rgay@mayo.edu.
³(Current Affiliation) Department of Orthopedic Surgery, Robert Debré Hospital, Paris, France

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fresh frozen specimens with the assumption that their mechanical properties reflect *in vivo* conditions. The effect of freezing on the mechanical properties of spinal specimens has been reported in several studies (Panjabi et al., 1985; Smeathers and Joanes, 1988; Bass et al., 1997; Pflaster et al., 1997; Gleizes et al., 1998; Dhillon et al., 2001). These studies have measured the changes in mechanical properties after a single occurrence of freezing. However, because many study designs require staged specimen preparation and testing, the effect of multiple freeze-thaw cycles on the mechanical properties of the spinal unit should be understood. We found no published papers addressing this issue. Therefore, our objective was to determine the effect of multiple freeze-thaw cycles on mechanical parameters measured during dynamic loading of porcine lumbar spines.

2. Methods

2.1 Specimen preparation

The lumbar spine was harvested from four female pigs immediately after sacrifice. All animals were obtained from studies approved by our Institutional Animal Care and Use Committee in accordance with the Federal Animal Welfare Act. Mean weight was 61.3kg and age 3 months. Non-ligamentous soft-tissues were removed leaving the vertebral bodies, discs, facet joints and ligamentous structures. Each spine was divided into two or three motion segments (vertebra-disc-vertebra) which provided 10 testable units (two each for L1/2, L2/3, L3/4, L4/5, L5/6). Motion segments were then potted in circular acrylic fixtures using polymethylmethacrylate and Kirshner wires. Specimens were kept moist with 0.9% saline-soaked toweling during testing (Wilke et al., 1998).

2.2 Biomechanical testing and storage

Specimens were tested in a custom dynamic spine testing apparatus described in a previous paper (Gay et al., 2006). Continuous pure moments were induced at 3 degrees/second to a limit of 5 Nm. Five cycles of flexion-extension, right and left lateral bending, and right and left rotation were performed and data from the 5th cycle was analyzed. At least five minutes were allowed for viscoelastic recovery between testing of different planes. All motion segments had baseline (BL) testing within 3 hours of sacrifice. They were then wrapped in toweling soaked with normal saline and stored in thick plastic bags at -20°C . After 48 to 72 hours they were thawed at room temperature (21°C), retested, and then refrozen. This process was repeated three times for a total of 3 post-thaw tests (PT1, PT2, and PT3).

Because specimens were exposed to a saline environment for a prolonged period during thawing, axial compression of 300 N was applied for 30 minutes before each post-thaw test session to minimize disc over-hydration. The cross sectional area of porcine discs has been shown to be approximately 35% of the human L3 vertebra (Berry et al., 1987). A 300 N load simulated the lumbar compression in a 70 kg man (980 N) standing upright (Nachemson, 1966).

2.3 Data analysis

Force, moment, and angular displacement data were recorded and moment-angle curves generated. A three-segment linear approximation was fit to each curve by a custom optimization program written in Matlab (The Mathworks, Natick, MA, USA) based on previous work (Markolf et al., 1976; Koff et al., 2006). The upper and lower segments of the curve were considered elastic zones (EZ) and the mid-portion the transitional zone (TZ). Range of motion (ROM), EZ size and slope, TZ size and slope, and the width of the hysteresis loop at the zero load position (HZ) were analyzed (Figure 1). We previously defined the TZ as a region of low resistance to dynamic motion around the neutral position (Gay et al., 2006). This concept is similar to the quasistatic neutral zone (Panjabi, 1992) which measures residual

deformation after static pure moment loading and therefore cannot be derived from continuous motion data. The study was designed to detect a 10% change in each parameter with power of 0.80 and alpha of 0.05 using repeated-measures ANOVA (effect = freeze-thaw cycle). Wilcoxon signed rank test for multiple comparisons was used to determine differences between pairs when statistical significance was found with ANOVA.

3. Results

Means and standard deviations of each parameter at all time points are presented in Table 1 and Table 2. The ROM was significantly decreased at PT1 compared to BL in flexion-extension (reduced to 93%) and lateral bending (reduced to 94%), however there was no difference between post-thaw measures (Figure 2). There was no significant change in rotation ROM.

The size of the TZ was significantly reduced in flexion-extension and lateral bending (reduced to 83% and 88% respectively) but not in rotation. The TZ size remained stable after the initial freeze-thaw cycle with no difference among post-thaw measures (Figure 2).

The TZ slope was significantly increased to 126 % in flexion-extension and to 122 % in lateral bending after freezing with no significant differences among post-thaw tests (Figure 3). The size and slope of the TZ did not change during axial rotation.

There was no significant change in the size of the HZ or in the size and slope of the EZ for any motion plane.

4. Discussion

We tested porcine lumbar motion segments after sacrifice and then after each of three freeze-thaw cycles. We found that parameters related to motion in the neutral region were changed by the initial freezing but not by subsequent freeze-thaw cycles.

The use of fresh frozen cadaver specimens is widely accepted in biomechanics research. Studies have described relatively small changes in material properties caused by frozen storage of ligament (Woo et al., 1986), tendon (Matthews and Ellis, 1968; Smith et al., 1996), muscle (Van Ee et al., 2000), disc annulus fibrosus (Nachemson, 1960), articular cartilage (Kiefer et al., 1989), cortical bone (Sedlin, 1965) and trabecular bone (Linde and Sorensen, 1993).

A single episode of freezing is thought to not affect some mechanical properties of human motion segments. These include the tensile properties of annulus fibrosus (Hirsch and Galante, 1967), hydrostatic pressure of the nucleus (Nachemson, 1960), ROM (Panjabi et al., 1985), and compressive stiffness and hysteresis (Smeathers and Joanes, 1988). In contrast, freezing does appear to affect porcine spines. The ultimate compressive load and energy absorbed to failure have been noted to increase (Callaghan and McGill, 1995). Additionally, compressive creep behaviors were altered by freezing (Bass et al., 1997). These studies evaluated the effects of a single freeze-thaw procedure; we could not find literature describing the effect of multiple freeze-thaw cycles.

We found that mechanical parameters related to stiffness in the neutral region (TZ size and slope) were changed by the initial freeze-thaw cycle but the EZ size and slope were not affected. Therefore, the decrease in TZ size was likely responsible for the observed decrease in ROM. Although previous spine studies did not specifically examine the effect of freezing on motion parameters in the neutral region, studies using arteries and ligaments have reported that a single freezing episode can affect stress-strain properties, particularly in low stress regions or the area of hysteresis (Woo et al., 1986; Venkatasubramanian et al., 2006).

Tissue water content has been implicated as an important factor determining deformation characteristics of biological materials. Permeability has been noted to increase in porcine discs after freezing while swelling pressure is decreased (Bass et al., 1997). Additionally, stiffness of motion segments tested in a saline bath was significantly lower than those tested in air (Costi et al., 2002). It is possible that our findings of decreased TZ size and increased TZ slope (stiffness) were due to changes in disc hydration caused by freezing. Because the specimens in the current study were exposed to saline over a prolonged period of time during thawing we chose to precondition them with axial compression before each post-thaw test session. We cannot be sure whether this had an effect on our results.

The difference between porcine and human spines should also be considered. Although freezing was found to alter the compressive creep behaviors of porcine spines (Bass et al., 1997), no effect was found on human spines using a similar technique (Dhillon et al., 2001). This difference might be due to higher water content in normal porcine discs, leading to increased ice crystal formation and nuclear expansion with freezing. Although the biomechanical response of the human spine to loading may not change as a result of freezing, further studies are needed to confirm this apparent difference.

In conclusion, we found that motion parameters that represent stiffness around the neutral position (TZ size and slope) in porcine motion segments were significantly affected by initial freezing but were not altered by as many as 3 subsequent freeze-thaw cycles. This suggests that porcine spine specimens that have been frozen and thawed multiple times can provide stable data in biomechanical testing.

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Figure 1.

Schematic representation of motion parameters from moment-displacement curves. ROM: range of motion, TZ: Transitional zone, HZ: width of Hysteresis loop, EZ: Elastic zone.

Figure 2.

Mean ($\pm$ SD) of the range of motion (ROM), size of elastic zone (EZ), and size of transitional zone (TZ) in flexion-extension.

Figure 3.

Mean ($\pm$ SD) of the slope of elastic zone (EZ) and transitional zone (TZ) in flexion-extension.

Table 1

Mean and standard deviation of motion parameters (degrees)

Parameters	Motion	BL	PT1	PT2	PT3	P-value
Size (°) ROM	Flex-Ext	14.6 ± 2.4	13.6 ± 2.5	13.8 ± 2.3	13.9 ± 2.3	p<0001
	Lat. bend	13.7 ± 2.1	12.9 ± 1.8	12.8 ± 1.9	12.9 ± 1.9	p<0001
	Rotation	6.1 ± 2.2	6.0 ± 2.2	6.1 ± 2.5	6.2 ± 2.2	N.S.
EZ	Flex-Ext	9.6 ± 0.9	9.4 ± 1.1	9.7 ± 1.3	9.8 ± 1.3	N.S.
	Lat. bend	7.2 ± 0.9	7.2 ± 0.9	7.1 ± 1.0	7.2 ± 0.9	N.S.
	Rotation	4.9 ± 1.3	4.8 ± 1.3	4.9 ± 1.0	5.2 ± 1.3	N.S.
TZ	Flex-Ext	5.0 ± 1.7	4.2 ± 1.6	4.1 ± 1.3	4.1 ± 1.2	p<0001
	Lat. bend	6.5 ± 1.4	5.7 ± 1.2	5.7 ± 1.2	5.7 ± 1.2	p<0001
	Rotation	1.2 ± 1.1	1.1 ± 1.1	1.3 ± 1.5	1.0 ± 1.1	N.S.
HZ	Flex-Ext	0.8 ± 0.4	0.8 ± 0.4	0.8 ± 0.7	0.8 ± 0.5	N.S.
	Lat. bend	1.7 ± 0.5	1.6 ± 1.1	1.6 ± 0.9	1.5 ± 0.8	N.S.
	Rotation	0.7 ± 0.5	0.7 ± 0.6	1.0 ± 1.1	0.8 ± 0.8	N.S.

BL=Baseline, PT =post-thaw conditions, P-values from repeated measures ANOVA

Table 2
Mean and standard deviation of the elastic zone (EZ) and transitional zone (TZ) slope (Nm/degrees)

Parameters	Motion	BL	PT1	PT2	PT3	P-value
Slope (Nm/degrees) EZ	Flex-Ext	0.97 ± 0.13	1.01 ± 0.16	0.97 ± 0.16	0.96 ± 0.17	N.S.
	Lat. bend	-1.59 ± 0.25	-1.57 ± 0.24	-1.62 ± 0.26	-1.57 ± 0.22	N.S.
	Rotation	2.14 ± 0.53	2.18 ± 0.53	2.15 ± 0.45	2.04 ± 0.51	N.S.
TZ	Flex-Ext	0.14 ± 0.06	0.18 ± 0.09	0.19 ± 0.09	0.19 ± 0.08	p=0.0007
	Lat. bend	-0.08 ± 0.03	-0.10 ± 0.04	-0.12 ± 0.04	-0.11 ± 0.04	p<0.0001
	Rotation	0.51 ± 0.34	0.57 ± 0.43	0.47 ± 0.43	0.5 ± 0.47	N.S.

BL=Baseline, PT =post-thaw conditions, P-values evaluated with repeated measures ANOVA.