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Abstract

To investigate the possible changes in material properties of cadaveric abdominal organs due to the preservation methods, the indentation data obtained from porcine abdominal organs (kidney, liver, and spleen) preserved by cooling and freezing are analyzed statistically in this study. Indentation tests were first conducted on fresh specimens. One half of the specimens of each organ were then frozen (preserved at −12 °C), and the other half of the specimens were cooled (preserved at 4 °C). All preserved specimens were retested after 20 days. Force and displacement data recorded during indentation were analyzed using a quasi-linear viscoelastic model. The results show that both cooling and freezing storage increased the kidney stiffness. In contrast, both storage methods decreased the stiffness of the spleen specimens. While cooling increased the liver stiffness, no significant changes of the instantaneous elastic response were observed in the liver specimens preserved by freezing. The liver and spleen's reduced relaxation responses and the liver's instantaneous elastic response were significantly different when comparing between cooling and freezing effects after 20 days of preservation. This study showed that both cooling and freezing storage methods significantly changed the material properties of abdominal organs, especially the instantaneous elastic response. More research is needed in investigating the effect of preservation on failure properties and mechanical properties under large deformation.

Keywords

Indentation, material properties, kidney, liver, spleen, tissue preservation

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Introduction

Abdominal injuries caused by traffic accidents have severe consequences and are major causes of death in the United States.[1–3] According to the National Automotive Sampling System (NASS) datasets, approximately 19,000 adult occupants sustain AIS 2 + abdominal injuries each year.[4] While the material properties of abdominal tissues were recently investigated, most of these studies[5–8] tested only fresh human or porcine abdominal organs. Since the majority of abdominal tests used to develop injury criteria were done with preserved cadavers,[9–13] there is a need to better understand possible changes to the material properties of the abdominal tissues caused by preservation methods (such as cooling or freezing).

During the past few years, human cadavers have served as invaluable tools for characterization of human biomechanical response during impact loading.[9] Therefore, development of reliable preservation methods for human cadavers, which minimize the biomechanical differences between living human and preserved cadavers, became very important for biomechanics research. During the late 1960s, it was shown that cadaver embalming significantly changed the response of human tissues, so testing of embalmed cadavers was abandoned.[14] After that, freezing and refrigeration methods were largely used to store human cadaveric specimens. Typically, the refrigeration method maintains the specimens at 4 °C–10 °C to slow tissue breakdown, and the freezer storage method preserves the subjects in frigid environments at temperatures

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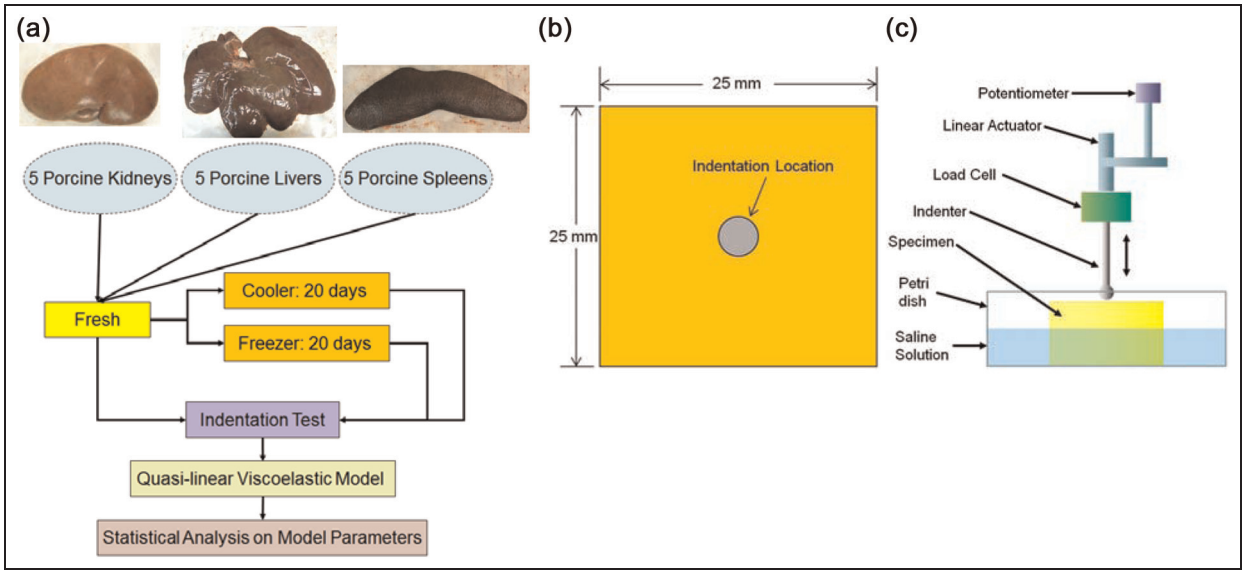

Figure 1. (a) Overall test procedure, (b) indentation location of each specimen, and (c) schematic illustration of the custom-built indentation-testing device used for experiments in the current study.

ranging from −10 °C to −70 °C. While several studies have reported some comparisons between fresh and frozen animal tissues in tensile tests,[15,16] the effect of cooling and freezing on the mechanical response of abdominal organs is still largely unknown.

The scope of this study was to investigate the influence of freezing and refrigeration storage methods on the biomechanical responses of abdominal tissues. The hypothesis in this study was that the material properties of the porcine abdominal organs change over time (20 days) for the specimens preserved by freezing and cooling. Indentation, a popular testing method that requires only a small volume of tissue and relatively easy sample preparation was used to find the local material properties of the tissues.[17,18] The test data recorded in typical relaxation tests and a quasi-linear viscoelastic (QLV) model were used to characterize the dynamic behavior of porcine abdominal tissues (kidney, liver, and spleen).

Methods

Specimen preparation and testing procedure

The overall testing procedure for the porcine abdominal soft tissues is shown in Figure 1(a). Intact fresh adult porcine abdominal organs, five kidneys, five livers, and five spleens, were obtained from a local slaughterhouse, saved in plastic bags covered by towels, and stored in an ice container with 4.5 kg ice during transportation until use. The direct contact between the organs and the ice was avoided. After arrival, each organ was sectioned into two equal halves: one half was used to investigate the freezing effect, and the other half was used to investigate the cooling effect. Four specimens ($25 \times 25 \times 15$ mm^3) were cut, using a custom blade assembly, from each half, keeping both the fibrous capsule and parenchyma intact and attached together. A total of 120 specimens were obtained (15 organs × 2 halves per organ × 4 specimens per half).

Indentation tests were first conducted on the capsule side of the porcine kidney, liver, and spleen specimens within 4 h of obtaining the tissues. Indentation ramp–hold tests with 1-mm displacement peaks (rate of indentation: 0.5 mm/s) and then a 2-min hold time were conducted on the center of each specimen (Figure 1(b)). A 250-g force uniaxial load cell (Honeywell Sensotec, Inc., Columbus, OH) was mounted between the linear actuator and the shaft of the 3.175-mm-radius spherical indenter tip (Figure 1(c)).[17] A linear potentiometer (model T-25; Novotechnik, Southborough, MA) was mounted on the actuator to verify the input displacement–time profile. Indenter–material contact was determined prior to each test by detection of a small (5 mN) load change. To maintain a consistent temperature during testing, all samples were immersed in physiological (0.9%) saline (Figure 1(c)).[7,8,19,20] The samples were rested on the petri dish during testing without flotation. The testing temperature was chosen to be close to a normal room temperature (24 °C), which approximates the post-mortem human surrogate (PMHS) temperature during abdominal tests. Four specimens from half of each organ were then frozen at −12 °C (freezing storage), and the four specimens of the other half were stored at 4 °C (cooling storage). All specimens were stored in the sealed container and under moist paper to ensure hydration, and then retested under the same testing condition and indentation locations after 20 days. This time interval approximates reasonably to the time required for performing the PMHS pretest medical examinations and obtaining the required approvals. The cooled and frozen specimens were rested in the saline before each test until a laser thermometer (model LT02; Maverick Industries, Inc.,

Edison, NJ) confirmed that the temperatures of the specimens were at 24 °C. This thawing process until testing took approximately 3 and 9 h for cooled specimens and frozen specimens, respectively. All fresh and preserved specimens were preconditioned (loading and unloading) by performing ramp–hold tests three times at a rate of 0.5 mm/s with 1 mm displacement peaks to reach the steady–state prior to the recording of force and displacement data during the fourth cycle.[21,22]

Material identification

The relationship between indentation force $P(t)$ and depth $h(t)$ was modeled using a QLV model

$$P(t) = \int_0^t G(t-\tau)\frac{\partial P^e(h)}{\partial h}\frac{\partial h}{\partial \tau}d\tau \quad (1)$$

where $P^e(h)$, representing the stiffness of a material, is the instantaneous elastic function, and $G(t)$ is the reduced relaxation function, and t is the time.[23] A discrete spectrum was assumed for the reduced relaxation function $G(t)$

$$G(t) = G_\infty + G_1 e^{-t/\tau_1} + G_2 e^{-t/\tau_2} + G_3 e^{-t/\tau_3} \quad (2)$$

subjected to the constraint $G(0) = G_0 = G_\infty + G_1 + G_2 + G_3 = 1$

where G_∞ is the long-time shear modulus ($G_\infty = \lim_{t\to\infty} G(t)$), and the G_i coefficients represent the relaxation strength corresponding to the τ_i time constant.[24] The contribution of the long-time shear modulus (G_∞) within the instantaneous shear modulus (G_0) can be simply represented by G_∞ (i.e. $G_\infty/G_0 = G_\infty$).[17]

The duration of the ramp was about 2 s and the hold time was 120 s as in the study of Mattice et al.[17] To reduce the number of unknown material parameters of the QLV model, three decay rates of relaxations ($1/\tau_i$) were assumed: $\tau_1 = 1$ s, $\tau_2 = 10$ s, and $\tau_3 = 100$ s.

The relationship between the instantaneous elastic function P^e and the indentation depth h was assumed to have a formula similar to the isotropic elastic Hertzian contact (spherical indentation) expression for an incompressible material[17]

$$P^e(\mathrm{t}) = \frac{8\sqrt{R}}{3}[2\mu_0]\cdot h(t)^{3/2} \quad (3)$$

where R is the indenter radius and μ_0 is the elastic shear modulus. The indentation force can then be described as

$$P(t) = \frac{16\sqrt{R}}{3}\int_0^t G(t-\tau)\left[\frac{d}{d\tau}\left(\mu_0 h(t)^{3/2}\right)\right]d\tau \quad (4)$$

The values of the reduced relaxation coefficients (G_∞, G_1, G_2, and G_3) and the elastic shear modulus (μ_0) (five optimization variables) were obtained by minimizing the sum of squared errors (SSEs) between the model and experimental forces using the active-set algorithm in MATLAB v. R2011b (The MathWorks, Inc., Natick, MA). This algorithm utilizes a sequential quadratic programming method used usually to solve medium-scale optimization problems (problems with reduced number of variables).[25] The initial values of G_∞, G_1, G_2, G_3, and μ_0 were chosen as 0.25, 0.25, 0.25, 0.25, and 5000 kPa, respectively. The time history of the indentor displacement recorded in testing was used in the calculation of the model indentation force (equation (4)). The averaged coefficient of determination (R^2) was calculated between the model and experimental forces for each treatment. G_∞ and μ_0, corresponding to the reduced relaxation response and instantaneous elastic response, served as indicators for comparing fresh and preserved tissues and comparing the cooled and frozen storage methods.

Statistical analysis

Paired two-sample t-test

To investigate the possible changes induced by cooling and freezing, the paired two-sample t-test was employed to compare material parameters (G_∞ and μ_0) between fresh and cooled tissues and between fresh and frozen tissues. A paired two-sample t-test is defined as

$$t = \frac{\bar{d}}{\sqrt{\frac{s^2}{n}}} \sim t_{df=n-1} \quad (5)$$

where $s^2 = \sum_{i=1}^{n}(d_i - \bar{d})^2/n - 1$, $\bar{d}$ is the mean difference between two measurements, and n is the sample size. The mean differences between two measurements tested at fresh and Day 20 were calculated, along with their corresponding p-values of the paired two-sample t-test. The critical α value was set to be 0.01.[26]

Generalized estimating equations statistical approach

In addition to the evaluation between the fresh and Day 20 tissues, a comparison between cooling and freezing effects was conducted using a statistical approach called generalized estimating equation (GEE). GEE is a robust statistical method employed to study population-average pattern or trend over time for longitudinal data.[27] In this study, the statistical model was expressed as $Y = \beta_0 + \beta_1 x_1 + \beta_2 x_1 x_2$, where Y is the material coefficients (G_∞ and μ_0). x_1 (the preservation time) and x_2 (the preservation method) are dummy (indicator) variables that indicate the presence or absence of categorical effect that may be expected to shift the outcome (Y). The x_1 dummy variable was assigned the values of 0 and 1, representing the fresh and preserved specimens, respectively. Similarly, the x_2 dummy variable was assigned the values of 0 and 1, representing the specimens preserved by freezing and cooling, respectively. Therefore, the outcomes, average

Table 1. Identified parameters of QLV material model (fresh vs preserved tissues).

	Cooling		Freezing	
	Fresh	Day 20	Fresh	Day 20
Kidney				
G_∞	0.237 ± 0.060	0.268 ± 0.049	0.267 ± 0.033	0.278 ± 0.040
G_1	0.490 ± 0.028	0.436 ± 0.048	0.478 ± 0.021	0.378 ± 0.032
G_2	0.105 ± 0.023	0.121 ± 0.019	0.115 ± 0.018	0.127 ± 0.024
G_3	0.167 ± 0.058	0.174 ± 0.031	0.141 ± 0.031	0.216 ± 0.024
μ_0 (kPa)	3.603 ± 0.504	4.626 ± 0.489	3.477 ± 0.472	4.674 ± 0.706
R^2	0.855	0.900	0.822	0.925
Liver				
G_∞	0.207 ± 0.059	0.153 ± 0.026	0.256 ± 0.055	0.231 ± 0.066
G_1	0.492 ± 0.042	0.547 ± 0.051	0.462 ± 0.030	0.474 ± 0.038
G_2	0.102 ± 0.026	0.153 ± 0.020	0.122 ± 0.014	0.095 ± 0.041
G_3	0.199 ± 0.060	0.147 ± 0.035	0.160 ± 0.052	0.200 ± 0.065
μ_0 (kPa)	3.406 ± 0.819	5.333 ± 1.349	3.930 ± 0.962	3.651 ± 0.708
R^2	0.796	0.927	0.799	0.809
Spleen				
G_∞	0.299 ± 0.037	0.214 ± 0.031	0.304 ± 0.049	0.256 ± 0.039
G_1	0.419 ± 0.039	0.513 ± 0.026	0.414 ± 0.040	0.443 ± 0.025
G_2	0.113 ± 0.013	0.118 ± 0.023	0.103 ± 0.013	0.136 ± 0.018
G_3	0.169 ± 0.017	0.156 ± 0.026	0.179 ± 0.029	0.165 ± 0.027
μ_0 (kPa)	7.882 ± 3.698	5.318 ± 1.776	7.068 ± 2.418	5.426 ± 0.965
R^2	0.956	0.914	0.953	0.941

Reported values are average ± 1 standard deviation.

values of material parameters (G_∞ and μ_0) recorded on the preserved specimens, can be expressed as

$$E[Y|frozen] = \beta_0 + \beta_1, \quad E[Y|cooling] = \beta_0 + \beta_1 + \beta_2 \tag{6}$$

The p-values of $\beta_2 = E[Y|cooling] - E[Y|frozen]$ less than .01 indicate that there are significant changes in terms of material properties between the specimens preserved by freezing and cooling. Both the paired two-sample t-test and GEE approach were conducted in SAS 9.2 (SAS Institute Inc., Cary, NC).

Results

Typical viscoelastic behaviors of abdominal organs recorded on specimens during ramp–hold indentation are presented in Figure 2. The average functions $P^e(h)$ and $G(t)$ corresponding to the three abdominal organs (kidney, liver, and spleen) and two different storage methods are shown in Figure 3. Identified parameters of the QLV material model are provided in Table 1. The unpaired two-sample t-test assuming equal variance was conducted on the fresh tissues in the two treatment groups, cooling and freezing, and it was proved that there were no significant differences of the fresh tissues on G_∞ and μ_0 between these two groups ($p > .01$).

The comparisons between the fresh and cooled tissues showed that μ_0 was significantly increased for kidney and liver ($p < .001$) while significantly decreased for spleen ($p < .001$) (Table 2). G_∞ was significantly decreased between fresh and cooled tissues for liver and spleen ($p < .001$). For the freezing effect, μ_0 was significantly increased for kidney ($p < .001$) while significantly decreased for spleen ($p = .001$). In addition, G_∞ was significantly decreased for spleen for the freezing effect ($p = .009$).

The differences between the cooling and freezing effects were investigated using the GEE statistical approach (Table 3). In liver, μ_0 after cooling was significantly higher by 1.991 kPa compared to that obtained by freezing ($p < .001$). For liver and spleen, G_∞ after cooling was significantly lower (5%–7%) than G_∞ after freezing ($p < .001$). Kidney's G_∞ and μ_0 and spleen's μ_0 were less sensitive to different treatments.

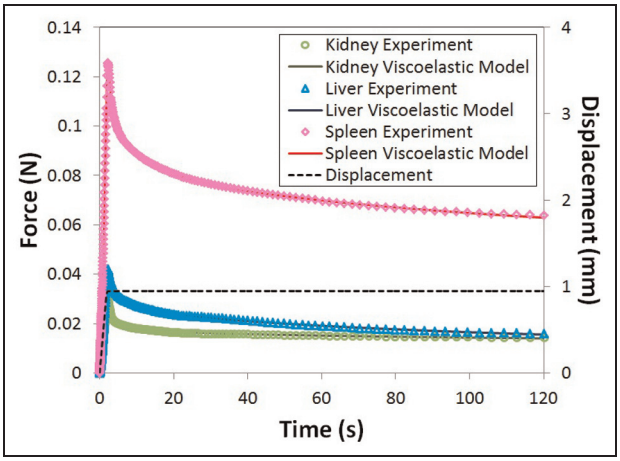

Figure 2. Examples of curve fitting with QLV model.

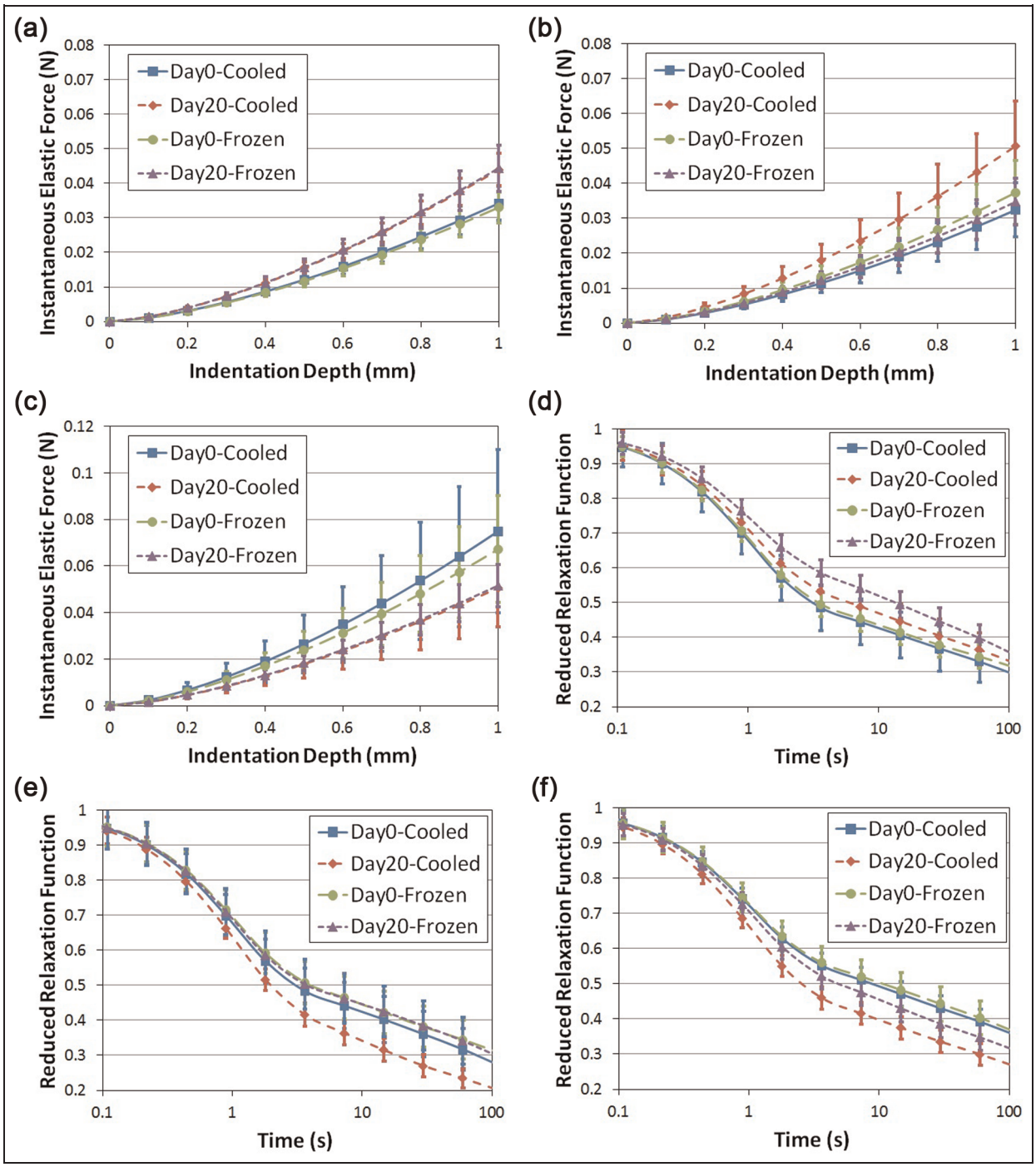

Figure 3. Average $P^e(h)$ for (a) kidney, (b) liver, and (c) spleen for the cooling and freezing storages and average $G(t)$ for (d) kidney, (e) liver, and (f) spleen for the cooling and freezing storages. Error bars represent ± 1 standard deviation.

Table 2. Comparison of fresh and preserved tissues for cooling and freezing storages using the paired two-sample t-test.

	Kidney		Liver		Spleen	
	Cooling	Freezing	Cooling	Freezing	Cooling	Freezing
G_∞	0.031 (.100)	0.011 (.281)	**−0.054 (<.001)**	−0.025 (.202)	**−0.085 (<.001)**	**−0.048 (.009)**
μ_0 (kPa)	**1.023 (<.001)**	**1.197 (<.001)**	**1.927 (<.001)**	−0.280 (.129)	**−2.564 (<.001)**	**−1.642 (.001)**

Boldfaced values represent the significant changes of coefficients ($\alpha = 0.01$).
Each cell represents the average of differences between fresh and preserved tissues of G_∞ and μ_0 and its corresponding p-value in the parentheses.

Table 3. Comparison between cooling and freezing storages for G_∞ and μ_0 using the GEE approach for kidney, liver, and spleen.

	Kidney	Liver	Spleen
G_∞	−0.009 (.525)	**−0.067 (<.001)**	**−0.045 (<.001)**
μ_0 (kPa)	−0.080 (.658)	**1.991 (<.001)**	−0.636 (.084)

Bolded values represent the significant β_2 ($\alpha = 0.01$).
Each cell represents the estimate of β_2 (Time–storage method interaction) and its corresponding p-value in the parentheses.

Discussion

The main hypothesis that the material properties of the porcine abdominal organs preserved by freezing and cooling change over time is supported by the results of this study in most circumstances. Overall, it could be observed that cooling increased the stiffness of kidney and liver but decreased the stiffness of spleen. Freezing increased the stiffness of kidney but decreased the stiffness of spleen.

Preservation by cooling and freezing storage showed an increase in the kidney stiffness, but no significant changes on its reduced relaxation function were found (Figure 3(a) and (d) and Table 2). Furthermore, GEE approach showed that both the reduced relaxation response and the instantaneous elastic response of the kidney were not sensitive when comparing the cooling to the freezing effects (Table 3). The increase of the kidney stiffness can be explained by the thick layer of the renal capsule. The renal capsule is composed of a tough fibrous layer, enriched with collagen and elastin (fibrous proteins), surrounding the kidney and covered in a thick layer of perinephric adipose tissue.[28] Previous studies have reported that the decomposition of elastin in vascular tissues during the periods of thawing at room temperature increased the stiffness in the toe region of the stress–strain curves.[29–31] This may suggest that a decomposition of elastin has occurred in the kidney capsule during the thawing process which made the organ more rigid. A similar increase in stiffness was not observed in the hepatic specimens due to their thinner capsule[32] and reduced content of elastin.[33] While the splenic capsule has higher content of elastin and showed an increased stiffness in tension after thawing,[33] a decrease of the instantaneous elastic response of splenic specimens in indentation was observed in the current study. This contradictory response suggests softening effects of the red pulp layer under the splenic capsule[34] caused probably by the preservation and the rapid autolysis process of the spleen.[35,36] Compressing the red pulp layer play an essential role in the response of the specimen during indentation, but have no or less effect on the tensile response of splenic tissues.

For the liver, the cooling effect made the liver stiffer while the freezing effect did not significantly change the instantaneous elastic response (Figure 3(b) and Table 2). Similarly, significant change on the liver's reduced relaxation function was observed by cooling storage, but this effect was not observed on liver specimens preserved by freezing (Figure 3(e) and Table 2). Both the reduced relaxation response and the instantaneous elastic response of the liver were highly sensitive when comparing the cooling to the freezing effects (Table 3).

Some previous studies reported similar findings to the liver stiffness responses: cooling made the liver stiffer[37] and freezing did not change the properties of the liver.[22] During the cooling process, increase of osmolality of the extracellular fluid of livers could be found, creating an osmotic gradient and causing the cells to dehydrate.[38,39] This may alter the structure of the liver cells while strengthening the elastic behaviors of the extracellular areas of the liver, leading to the increase of the stiffness of the tissues. This result was comparable to those reported by the study of Ocal et al.,[37] who tested bovine livers in several preselected time points within 48 h after harvesting. On the other hand, while both extracellular and intracellular ice formation could be achieved with fast freezing or freezing to very low temperatures,[40] it has been shown that cells may survive freezing and rehydrate after thawing.[41–43] Porcine liver parenchyma exhibits better recovery ability and therefore has no considerable difference in the compressive response of the fresh versus previously frozen samples, as demonstrated in Tamura et al.[22] While the liver properties may not be influenced by the freezing effect in terms of the compressive response, Santago et al.[16] commented that any cellular damage that might have been caused by the freezing process may have effect on the failure property of the soft tissue, according to their tensile testing study on bovine livers. In addition, Brunon et al.[44] indicated that the freezing preservation may affect the failure properties of the porcine liver capsule alone. While the current study quantifies only the material properties under the indentation testing, further comparison of the storage methods and the failure properties between different loading types would be suggested.

Both the cooling and freezing storage methods softened the spleen specimens (Figure 3(c) and Table 2). While after 20 days, the spleen's reduced relaxation response preserved by cooling was significantly lower than those preserved by freezing ($p < .001$), its instantaneous elastic response was not significantly different when comparing these two preservation methods ($p = .084$) (Table 3). These observations can be explained by the rate of the autolysis in the splenic cellular composition and architecture. The capsule of the spleen is relatively homogeneous and is composed of collagen, elastic fibers, and smooth muscles, while the parenchyma of the spleen is less homogeneous and consists of white and red pulps, vasculature, and trabeculae.[28] Autolysis is the destruction process of a cell through the action of its own enzymes that eventually softens the organs.[28] At the normal room temperature, the symptoms of the autolysis occur in spleen within 17 h after the death,[35] resulting, generally, in a poor

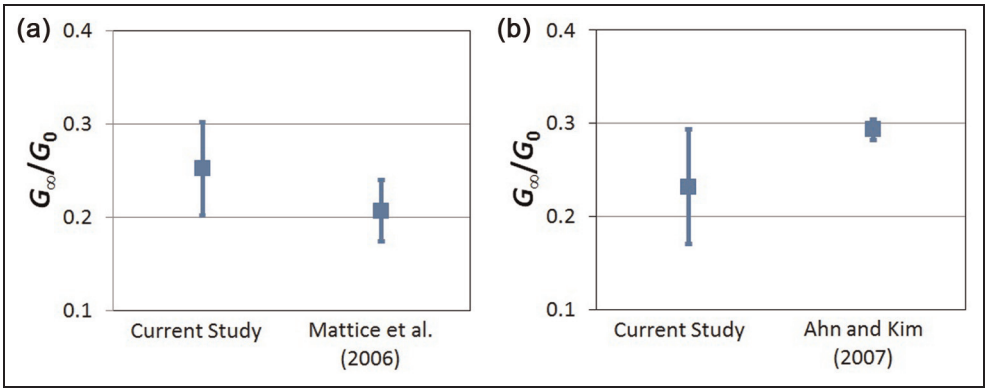

Figure 4. Comparison of the shear modulus ratios with published studies for (a) fresh porcine kidney and (b) fresh porcine liver.

morphological preservation of the spleen.[36] For the kidney and liver preserved at the room temperature, the symptoms of the autolysis usually occur within 36 h after the death.[28] It is also well-known that the rate of the autolysis can be decreased by low temperature.[28] These data suggest that the spleen gradually became softer before the low temperature storage and had faster autolysis rate than kidneys and livers during the cooling or freezing preservation.

In general, the mean values of the ratios of the long-time shear modulus (G_∞) to the instantaneous shear modulus (G_0) for both fresh porcine kidney and liver were close to the values reported in the previous studies,[17,45] but some differences were also observed. The unpaired two-sample t-test assuming unequal variance showed that this ratio for fresh porcine kidney obtained in the current study was significantly higher than the ratio obtained in the study of Mattice et al.[17] ($p = .001$) (Figure 4(a)), while this ratio for fresh porcine liver obtained in the current study was significantly lower than the ratio obtained in the study of Ahn and Kim[45] ($p < .001$) (Figure 4(b)). These discrepancies could be caused by different viscoelastic modeling techniques. Mattice et al.[17] utilized linear viscoelastic models, while Ahn and Kim[45] utilized only two time constants (average $\tau_1 = 1.136$ and $\tau_2 = 51.204$).

Similar biomechanical behaviors between human and porcine abdominal organs have been reported in some previous studies (e.g. kidney,[8] liver,[5] and spleen[46]). In fact, Vodicka et al.[47] showed that the miniature pig, sharing many physiological similarities with humans, offered several breeding and handling advantages (when compared to nonhuman primates), making it an optimal species for preclinical experimentation. Thus, the porcine abdominal tissues could serve reasonably as a surrogate of human tissues. However, there is a lack of data regarding the comparison of the tissue properties between different storage conditions among the porcine and human abdominal tissues, and the comparison between the in vivo and in situ properties is unknown; therefore, these comparisons could be further investigated.

One assumption in the indentation test is the isotropy of the tissues; however, biological tissues usually exhibit anisotropic properties. Farshad et al.[48] studied the uniaxial compression behavior of porcine kidney parenchyma sample at various loading speeds and showed that the parenchyma tissue was not only rate dependent, but also anisotropic. In addition, Chui et al.[5] found that with the primary axis perpendicular to the cross-sectional surface of porcine liver tissue specimens, the tissue was stiffer with tensile or compressive force in the axial direction compared to that of the transverse direction. Therefore, the investigation of the isotropic behaviors of the abdominal organs under the cooling and freezing effects by indenting different directions on the tissues would be suggested to acquire the full scope of the tissue mechanical responses. In addition, since several studies (e.g. Bass et al.[49]) showed that the mechanical properties of soft tissues are temperature dependent, additional experiments should be performed to investigate the effect of preservation on abdominal tissues conducted at different temperatures (e.g. body temperature vs room temperature).

In this study, specimens with connected outer capsule and inner parenchyma structures were tested, and the capsule side was loaded rather than the parenchyma side, as shown in other studies testing on porcine abdominal tissues.[5,50,51] However, some previous studies have shown the different material properties of the capsule and parenchyma.[5,8,19,51] Therefore, the effects of degradation over time may be underestimated in this study because the capsule is much stronger and more resilient than the parenchyma for the abdominal tissues due to the collagen structure of the capsule, so its properties may not be changed largely by the cooling and freezing effects in comparison with the parenchyma. Testing these structures individually will be desirable, and the results can be more accurate to model the mechanical behavior of abdominal organ components (e.g. in finite element models) and then develop smart restraint systems.[52,53,54]

While the indentation-based properties may be within the toe region of the entire stress–strain curve of

abdominal organs, additional tests (using other testing methods) incorporating the effects of preservation methods in the domain of higher strains would help to better understand the tissue behavior in the failure region.

Conclusions

This study shows that the cooling effect impacted the instantaneous elastic response of all three organs but changed relaxation response for liver and spleen only. In contrast, the freezing effect changed the instantaneous elastic response and the reduced relaxation response of kidney and spleen, except kidney's reduced relaxation response. Statistical analysis found that there were significant differences on the material properties of the liver and spleen between cooling and freezing methods, but there was no difference between the cooling and freezing effects in the kidney. These material properties could be further combined with finite element simulations to generate a better understanding of the effect of these material changes on the results of PMHS tests under abdominal loading.

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References

1. Arajärvi E, Santavirta S and Tolonen J. Abdominal injuries sustained in severe traffic accidents by seatbelt wearers. *J Trauma* 1987; 27(4): 393–397.
2. Cheynel N, Gentil J, Freitz M, et al. Abdominal and pelvic injuries caused by road traffic accidents: characteristics and outcomes in a French cohort of 2,009 casualties. *World J Surg* 2011; 35(7): 1621–1625.
3. Greingor JL and Lazarus S. Chest and abdominal injuries caused by seat belt wearing. *South Med J* 2006; 99(5): 534–535.
4. Klinich KD, Flannagan CAC, Nicholson K, et al. *Abdominal injury in motor-vehicle crashes*. University of Michigan Transportation Research Institute, Report No: UMTRI-2008-40, November 2008.
5. Chui C, Kobayashi E, Chen X, et al. Transversely isotropic properties of porcine liver tissue: experiments and constitutive modeling. *Med Biol Eng Comput* 2007; 45: 99–106.
6. Kemper AR, Santago AC, Stitzel JD, et al. Biomechanical response of human spleen in tensile loading. *J Biomech* 2012; 45(2): 348–355.
7. Snedeker JG, Barbezat M and Niederer P. Strain energy density as a rupture criterion for the kidney: impact tests on porcine organs, finite element simulation, and a baseline comparison between human and porcine tissues. *J Biomech* 2005; 38(5): 993–1001.
8. Snedeker JG, Niederer P and Schmidlin FR. Strain-rate dependent material properties of the porcine and human kidney capsule. *J Biomech* 2005; 38(5): 1011–1021.
9. Crandall JR, Bose D, Forman J, et al. A review of human surrogates for injury biomechanics research. *Clin Anat* 2011; 24(3): 362–371.
10. Eichberger A, Darok M, Steffan H, et al. Pressure measurements in the spinal canal of post-mortem human subjects during rear-end impact and correlation of results to the neck injury criterion. *Accid Anal Prev* 2000; 32(2): 251–260.
11. McIntosh AS, Kallieris D and Frechede B. Neck injury tolerance under inertial loads in side impacts. *Accid Anal Prev* 2007; 39(2): 326–333.
12. Salzar RS, Bass CR, Lessley D, et al. Viscoelastic response of the thorax under dynamic belt loading. *Traffic Inj Prev* 2009; 10(3): 290–296.
13. Untaroiu CD, Bose D, Lu Y-C, et al. Effect of seat belt pretensioners on human abdomen and thorax: biomechanical response and risk of injuries. *J Trauma Acute Care Surg* 2012; 72(5): 1304–1315.
14. Crandall JR. *Preservation of human surrogates for impact studies*. PhD Thesis, University of Virginia, Charlottesville, VA, 1994.
15. Lee KE and Pelker RR. Effect of freezing on histologic and biomechanical failure patterns in the rabbit capital femoral growth plate. *J Orthop Res* 1985; 3: 514–515.
16. Santago AC, Kemper AR, McNally C, et al. Freezing affects the mechanical properties of bovine liver. *Biomed Sci Instrum* 2009; 45: 24–29.
17. Mattice JM, Lau AG, Oyen ML, et al. Spherical indentation load-relaxation of soft biological tissues. *J Mater Res* 2006; 21(8): 2003–2010.
18. Van Dommelen JA, Van der Sande TP, Hrapko M, et al. Mechanical properties of brain tissue by indentation: interregional variation. *J Mech Behav Biomed Mater* 2010; 3: 158–166.
19. Davies PJ, Carter FJ, Roxburgh DG, et al. Mathematical modeling for keyhole surgery simulations: spleen capsule as an elastic membrane. *Theor Med* 1999; 1(4): 247–262.
20. Manoogian SJ, Bisplinghoff JA, McNally C, et al. Effect of strain rate on the tensile material properties of human placenta. *J Biomech Eng* 2009; 131(9): 091008.
21. Shafieian M, Darvish KK and Stone JR. Changes to the viscoelastic properties of brain tissue after traumatic axonal injury. *J Biomech* 2009; 42(13): 2136–2142.
22. Tamura A, Omori K, Miki K, et al. Mechanical characterization of porcine abdominal organs. *Stapp Car Crash J* 2002; 46: 55–69.
23. Fung YC. *Biomechanics: mechanical properties of living tissues*. 2nd ed. New York: Springer, 1993.
24. Troyer KL, Estep DJ and Puttlitz CM. Viscoelastic effects during loading play an integral role in soft tissue mechanics. *Acta Biomater* 2012; 8: 234–243.
25. Coleman T. *MATLAB optimization toolbox user's guide v. R2011b*. Natick, MA: The MathWorks, Inc., 2011.
26. Milton JS and Arnold J. *Introduction to probability and statistics: principles and applications for engineering and the computing sciences*. 4th ed. New York, NY, USA, McGraw-Hill, 2003.
27. Fitzmaurice GM, Laird NM and Ware JH. *Applied longitudinal analysis*. 2nd ed. Hoboken, NJ, USA, John Wiley & Sons, 2004.

28. Fawcett DW. *Bloom and Fawcett: a textbook of histology*. 11th ed. Philadelphia, PA: W.B. Saunders Company, 1986.
29. Gundish N. *Role of elastin and collagen in the passive mechnics of the circulatory system*. PhD Thesis, University of California, Berkeley, CA, 2004.
30. Roach MR and Burton AC. The reason for the shape of the distensibility curves of arteries. *Can J Biochem Physiol* 1957; 35(8): 681–690.
31. Venkatasubramanian RT, Grassl ED, Barocas VH, et al. Effects of freezing and cryopreservation on the mechanical properties of arteries. *Ann Biomed Eng* 2006; 34(5): 823–832.
32. Umale S, Deck C, Bourdet N, et al. Experimental mechanical characterization of abdominal organs: liver, kidney and; spleen. *J Mech Behav Biomed Mater*, 2012; http://dx.doi.org/10.1016/j.jmbbm.2012.07.010.
33. Nguyen NH, Duong MT, Tran TN, et al. Influence of a freeze-thaw cycle on the stress-stretch curves of tissues of porcine abdominal organs. *J Biomech* 2012; 45(14): 2382–2386.
34. Schuenke M, Schulte E, Schumacher U, et al. *Atlas of anatomy: neck and internal organs*. New York, NY, USA, Thieme, 2006.
35. Corper HJ. Correlation of the histological and chemical changes in the spleen during necrosis and autolysis. *J Exp Med* 1912; 15(5): 429–449.
36. Wilkins BS and Wright DH. *Illustrated pathology of the spleen*. 1st ed. Cambridge, UK, Cambridge University Press, 2000.
37. Ocal S, Ozcan MU, Basdogan I, et al. Effect of preservation period on the viscoelastic material properties of soft tissues with implications for liver transplantation. *J Biomech Eng* 2010; 132(10): 101007.
38. Gill W and Fraser I. A look at cryosurgery. *Scott Med J* 1968; 13(8): 268–273.
39. Whittaker DK. Mechanisms of tissue destruction following cryosurgery. *Ann R Coll Surg Engl* 1984; 66(5): 313–317.
40. Mazur P. The role of intracellular freezing in the death of cells cooled at supraoptimal rates. *Cryobiology* 1977; 14(3): 251–272.
41. Farrant J and Walter CA. The cryobiological basis for cryosurgery. *J Dermatol Surg Oncol* 1977; 3(4): 403–407.
42. Jacob G, Kurzer MN and Fuller BJ. An assessment of tumor cell viability after in vitro freezing. *Cryobiology* 1985; 22(5): 417–426.
43. Shier WT. Studies on the mechanisms of mammalian cell killing by a freeze-thaw cycle: conditions that prevent cell killing using nucleated freezing. *Cryobiology* 1988; 25(2): 110–120.
44. Brunon A, Bruyère-Garnier K and Coret M. Mechanical characterization of liver capsule through uniaxial quasi-static tensile tests until failure. *J Biomech* 2010; 43(11): 2221–2227.
45. Ahn B and Kim J. An efficient soft tissue characterization method for haptic rendering of soft tissue deformation in medical simulation. In: *Proceedings of the 2007 frontiers in the convergence of bioscience and information technologies*, October 11–13, 2007, Jeju Island, Korea, pp.549–553, IEEE.
46. Carter F, Russell E, Dunkley P, et al. Restructured animal tissue model for training in laparoscopic anti-reflux surgery. *Minim Invasiv Ther* 1994; 3(2): 77–80.
47. Vodicka P, Smetana K, Dvoránková B, et al. The miniature pig as an animal model in biomedical research. *Ann NY Acad Sci* 2005; 1049: 161–171.
48. Farshad M, Barbezat M, Flüeler P, et al. Material characterization of the pig kidney in relation with the biomechanical analysis of renal trauma. *J Biomech* 1999; 32(4): 417–425.
49. Bass CR, Planchak CJ, Salzar RS, et al. The temperature-dependent viscoelasticity of porcine lumbar spine ligaments. *Spine (Phila Pa 1976)* 2007; 32(16): E436–E442.
50. Gao Z, Lister K and Desai JP. Constitutive modeling of liver tissue: experiment and theory. *Ann Biomed Eng* 2009; 38(2): 505–516.
51. Seki S and Iwamoto H. Disruptive forces for swine heart, liver, and spleen: their breaking stresses. *J Trauma* 1998; 45(6): 1079–1083.
52. Adam T and Untaroiu CD. Identification of occupant posture using a Bayesian classification methodology to reduce the risk of injury in a collision. *Transport Res C: Emer* 2011; 19(6): 1078–1094.
53. Untaroiu CD and Adam T. Performance-based classification of occupant posture to reduce the risk of injury in a collision. *IEEE T Intell Transp*, DOI: 10.1109/TITS.2012.2223687.
54. Fredriksson R, Shin J, Untaroiu, CD. Potential of Pedestrian Protection Systems – A Numerical Parameter Study Using Finite Element Models of a Pedestrian Dummies and Generic Sedan Vehicles, *Traffic Injury Prevention* 20011; 12(4): 398–411.