Influence of refrigerated storage on tensile mechanical properties of porcine liver and spleen

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Preservation conditions may affect internal organs, thus influencing the results of protracted biomechanical tests. The influence of the freeze–thaw cycle on the mechanical behaviour of porcine abdominal organs was reported in our previous work. Here, we further investigate the effects of refrigerated storage on the mechanical properties of porcine spleen and liver. Twenty-seven swine were chosen for tensile testing. To simulate the conservation conditions before biomechanical experimentation, the total sample was divided into three groups of nine individuals each and tested in a fresh state, after a freeze–thaw cycle, and after refrigerated storage (+4 °C). Fitted stress–stretch curves for each tissue type were obtained by employing a modified Fung model for isotropic behaviour. The results suggest statistically significant effects of refrigerated storage on the spleen but negligible influence on the liver. Similarly to the impact of the freeze–thaw cycle, refrigerated storage caused a decrease in the mechanical properties of the spleen. This again supports the hypothesized cause of the altered behaviour of spleen due to the autolysis of elastin by elastolytic enzymes during and after the cooling period. Spleen and liver are good examples of tissue with and without elastin. These findings have wide biomechanical and decomposition implications for the study of soft tissues.

Keywords: refrigerated storage; freeze–thaw; autolysis; liver; spleen

1. Introduction

Abdominal solid organs are frequently injured in both front and side impact collisions from traffic accidents (Franklyn et al. 2002). Important improvements of vehicle safety have been achieved with the help of post-mortem human surrogate (PMHS) and human volunteer tests as well as simulations with human models (rigid-body and finite element model) (Foster et al. 2006; Untaroiu et al. 2012). The societal acceptance of using PMHS varies with regional culture, habits, traditions and religions. On the other hand, the swine anatomy is reasonably comparable to the human anatomy (Ibrahim et al. 2006), especially considering the organ structure and function.

Contrary to in vivo testing, tissue is often tested ex corporally and may be subjected to changes in mechanical properties after preservation (Stemper et al. 2007; Nguyên et al. 2012). These effects can be significant if the tissue undergoes different preservation conditions or is not tested in a fresh state. It has been found that preservation by a freeze–thaw process shifts the mechanical behaviour from higher to lower maximum stretch in the porcine spleen, but has only negligible impact on the liver (Nguyen et al. 2012). Similarly, no noticeable differences were found between frozen (for 24 h then thawed) and fresh porcine liver (Tamura et al. 2002). In contrast, Lu et al. (2014) claimed that freezing significantly affects the parenchyma in the bovine liver from reduced stretch, whereas stress failure and loading rate increased concurrently. Freezing also changed the material properties of porcine arteries (Venkatasubramanian et al. 2006) and bovine thoracic aortas (Chow & Zhang 2011). Moreover, Temifi et al. (2013) suggest that kidney tissue should not be frozen prior to biomechanical characterization due to significant decrease in shear modulus. Therefore, freeze–thaw consequences are different depending on experiments (liver capsules or liver capsule with parenchyma) conducted for various preservation conditions and loadings. In this paper, freeze storage was also alternatively applied in order to compare its effects quantitatively with those caused by the cooling technique for porcine abdominal organs.

Besides the influence of freezing and thawing, cooling preservation can cause significant changes in the mechanical response of tissues. Generally, cool storage of tissue at +4 °C for short and long durations is adopted by many experiments. For short periods, researchers have employed this method to minimize post-mortem decomposition. Refrigerated storage for a short duration (intermediate storage is often smaller than 6 h) is often utilized, but its influence has mostly not been considered; usually tissue preserved in this manner is considered as fresh (Nicolle & Palierne 2010; Nicolle et al. 2012).

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Cold storage of bovine arteries at +4 °C tested in a biaxial setup was reported by Chow and Zhang (2011). This preservation method significantly decreases the initial stiffness in the toe region but increases the slope in the linear part of the stress–strain curve. Stemper et al. (2007) observed that refrigerated porcine arterial tissue at +4 °C after 24 and 48 h showed changes in mechanical properties, decreases in ultimate stress and Young’s modulus, but no difference between the frozen (−80 °C) and the fresh arteries was seen. They suggested that the effects were due to tissue degeneration during refrigerated storage. In other tests, refrigerated storage (+4 °C) affects a mouse carotid artery resulting from a splenectomy had been performed, a localized liver injury was induced in a highly reproducible manner with a clamp. It was possible to finish the tests of fresh spleen tissue in a neighbouring operation room before the liver was extracted post-mortem and prepared for tension tests without any delay. All organs were harvested with major blood vessels intact and tested extracorporeally. Specimens were only cut from unwounded parts of the liver. The previous tests protocols of fresh and frozen organs (Nguyén et al. 2012) were repeated for new animals in order to increase data and confidence. The organs were divided into three groups: nine animals tested in fresh state, another nine subjected to the freeze–thaw cycle, and the remaining nine cooled in a cooling chamber at +4 °C. The fresh organs were brought to the laboratory within 15 min. Each ex vivo test was completed within 4 h of organ retrieval; each specimen was kept moist with standard saline during the experiments. No swine was sacrificed for this project.

The organs subjected to a freeze–thaw cycle were frozen at −18 °C. Herein, we considered the decomposition processes as a cycle of storage at room temperature (RT = +20 °C to +25 °C, wetted with normal saline solution), freezing, thawing, storage in a refrigerator and finally at RT again. The applied temperature settings and times may be seen in Table 1, which shows the basic cycle of freezing the organs 6 h after harvesting. All organs were subjected to the identical temperature protocols and time intervals.

The nine abdominal organs, covered with the intact intestine and abdominal wall of the same animal, were incubated in saline solution at +4 °C in a cooling chamber for one week and tested within 4 h of removal, as illustrated in Figure 1. This procedure has been designed to mimic intra-abdominal situation during the storage of PMHS before biomechanical injury without the need to store whole animals for one week. The spleen organs were only tested along the longitudinal direction due to insufficient dimension in the transverse direction, whereas the tests of liver organs were conducted along the longitudinal directions and the transverse directions of their lobes. The lower face of the spleen was not used for experiments due to a density of arteries and veins. In contrast, both the lower and upper faces of the liver were used. All specimens were cut into a rectangular shape of 50–65 mm long and 15 mm wide. Specimen thickness was smaller than <2.5 mm (including capsule and parenchyma for both liver and spleen. It is difficult to remove parenchyma in spleen capsule specimens). A single-column testing machine Zwick/Roell Z0.5 with a pre-load of 0.2 N was used. The free test length of the

2. Methods
2.1. Preparation of specimens
Organs were taken of 27 three-month-old pigs (n = 27) with an average weight of 35.13 ± 5.12 kg (mean ± SD) which were anesthetized and operated under appropriate animal care protocols in an operating room at the University Hospital, RWTH Aachen, Germany. As detailed in (Grottker et al. 2010), after a splenectomy had been performed, a localized liver injury was induced in a highly reproducible manner with a clamp. It was possible to finish the tests of fresh spleen tissue in a neighbouring operation room before the liver was extracted post-mortem and prepared for tension tests without any delay. All organs were harvested with major blood vessels intact and tested extracorporeally. Specimens were only cut from unwounded parts of the liver. The previous tests protocols of fresh and frozen organs (Nguyén et al. 2012) were repeated for new animals in order to increase data and confidence. The organs were divided into three groups: nine animals tested in fresh state, another nine subjected to the freeze–thaw cycle, and the remaining nine cooled in a cooling chamber at +4 °C. The fresh organs were brought to the laboratory within 15 min. Each ex vivo test was completed within 4 h of organ retrieval; each specimen was kept moist with standard saline during the experiments. No swine was sacrificed for this project.

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specimens was 24 mm. Tests were done with the strain rate of 0.083 s\(^{-1}\).

### 2.2. Material model

The purpose was to investigate the influence of the preservations on tissue elasticity, particularly in uniaxial tension tests for the spleen and liver. The liver and the spleen are assumed to be isotropic. Thus, a modified Fung formulation (Duong et al. 2012; Nguyễn et al. 2012) for isotropic materials was adopted for parameter identification expressed as

\[
W(E) = C(\exp(Q) - 1),
Q = A_{11}(E_{11}^2 + E_{22}^2 + E_{33}^2) + 2 A_{12}(E_{11}E_{22} + E_{11}E_{33} + E_{22}E_{33}),
\]

where \(C\) has the dimension of a modulus; \(A_{11}\) and \(A_{12}\) are dimensionless coefficients. In the form of Voigt’s notation, the Green–Lagrange strain and the second Piola–Kirchhoff stress are written as the matrices

\[
E = [E_{11}, E_{22}, E_{33}, 2E_{12}, 2E_{23}, 2E_{31}]^T
\]

and

\[
\Sigma = [S_{11}, S_{22}, S_{33}, S_{12}, S_{23}, S_{31}]^T,
\]

respectively. The stiffness at the end of the stress–stretch curve (caused mainly by collagen fibre) was not considered since the cause of change in this study was supposed to be the change through elastin, at lower stretch. Thus, the initial tangent stiffness of the stress–stretch curve (Nguyễn et al. 2012) is defined as

\[
k_0 = \frac{\delta \sigma}{\delta E_{11}}\bigg|_{E_{11}=0} = 2CA_{11}.
\]

#### 2.2.1. Stress and stretch measures in uniaxial tension tests

The uniaxial stress, see Figure 1(b):

\[
\sigma = [\sigma, 0, 0, 0, 0, 0]^T, \quad \Sigma = [\Sigma, 0, 0, 0, 0, 0]^T,
\]

where \(\sigma\) is the true Cauchy stress and \(\Sigma\) is the nominal stress which is called first Piola–Kirchhoff stress in continuum mechanics. Then the deformation gradient \(F\) and the Green strain \(E\) in Voigt’s form are

\[
F = \left[\lambda, \lambda^{-\frac{1}{2}}, \lambda^{-\frac{1}{2}}, 0, 0, 0\right]^T;
\]

\[
E = \frac{1}{2}\left[(\lambda^2 - 1), (\lambda^{-1} - 1), (\lambda^{-1} - 1), 0, 0, 0\right]^T,
\]

### Table 1. Freeze–thaw cycle for abdominal organs.

| Time          | Period | Temperature (°C) | Period (h) | Temperature (°C) |
|---------------|--------|-----------------|------------|-----------------|
| ↓             | 6 h    | +21             | 6          | +20 to +25      |
| Some months   |        | −21             | >20        | −18 to −2       |
| 3–4 days      |        | +21             | 12         | +20 to +25      |
| 5–7 h         |        | +4              | 3          | +4 to +8        |
| ~1 h          |        | +18 to +25      |            |                 |
| ~11 h         |        | +4              |            |                 |
| ~7–9 h        |        | +19 to +23      | 1          | +20 to +25      |

Figure 1. Cooling preservation and uniaxial tension test of spleen.

(a) Organs covered by intestine and abdominal wall (removed for photo) in cooling chamber

(b) Uniaxial tension test
where the stretch \( \lambda = \frac{l}{L} \) is defined as the ratio between deformed length \( l \) and undeformed length \( L \).

The stress \( \Sigma \) is defined with load \( f \) and undeformed area \( A_0 \) as

\[
f = \Sigma A_0 \Rightarrow \Sigma = \frac{f}{A_0}. \tag{5}
\]

By the incompressibility condition (deformed area \( A = \frac{1}{\lambda^2} A_0 \)), the Cauchy stress is evaluated as

\[
f = \sigma A = \sigma \frac{1}{\lambda} A_0 \Rightarrow \sigma = \frac{f}{A_0} = \lambda \Sigma. \tag{6}
\]

### 2.2.2. Failure measures

The ultimate tensile Cauchy stresses \( \sigma_{ult} \) of the tissues in tensile tests were computed from the experimentally determined fracture forces according to

\[
\sigma_{ult} = \frac{F}{A_0} \lambda_{ult}, \tag{7}
\]

where \( F \) is the tensile fracture force and \( \lambda_{ult} \) is the ultimate tensile stretch.

### 2.3. Material parameter identification

The coefficients of the modified Fung model presented above \((C, A_{11} \text{ and } A_{12})\) were optimized in a curve-fitting process performed on sets of data points of average stress–stretch curves (obtained from experimental data) by using non-linear least squares algorithms in Matlab (MATLAB R2010a, The Mathworks, Inc., Natick, MA), such as a subspace trust region method that is based on the interior-reflective Newton method to minimize the function

\[
\sigma_{error}(C,A_{11},A_{12}) = \frac{1}{2} \sum_{i=1}^{n} [\sigma^e_i(C,A_{11},A_{12}) - \sigma^f_i]^2, \tag{8}
\]

where \( \sigma^e_i \) is the stress of data point \( i (i = 1,\ldots,n) \) of the experimental stress–stretch curve as in (6), where as \( \sigma^f_i(C,A_{11},A_{12}) \) is the Cauchy stress calculated from the modified Fung model (1) in the uniaxial tension test as

\[
\sigma^f = \lambda^2 S_{11} - \lambda^{-1} S_{22}, \tag{9}
\]

where

\[
S_{11} = \frac{\delta W}{\delta E_{11}} = 2C[A_{11}E_{11} + A_{12}(E_{22} + E_{33})] \exp(Q), \tag{10}
\]

\[
S_{22} = \frac{\delta W}{\delta E_{22}} = 2C[A_{12}E_{22} + A_{11}(E_{11} + E_{33})] \exp(Q). \tag{11}
\]

In the curve-fitting process, the initial values of \( C, A_{11} \) and \( A_{12} \) were imposed as 1.0 MPa, 0.6 and 0.5, respectively. The average stress–stretch curves utilized in the fitting were calculated from the experimental data by employing a normalization technique (Lessley et al. 2004). In this method, the average curves and their variation corridors were computed. Moreover, we also applied an elliptical corridor approach to represent the variability in both the \( x \)- and \( y \)-coordinates along the average curve (Untariou & Lu 2013; Untariou et al. 2015). Therefore, the corridors were defined as they envelop the ellipsoids along the average curve with the ellipsoid axes denoting the corresponding standard variations, see Figures 3 and 4.

### 2.4. Statistical analysis

The effects of the preservation methods on the biomechanical properties of porcine abdominal organs are examined by analysing the statistical significance of group differences using a one-way analysis of variance (ANOVA) at the level of significance \( \alpha = 0.05 \). The null hypothesis was that the mean was the same for all groups. The ultimate stress and stretch of each group were statistically analysed. We also distinguished among the groups which pairs of means were significantly different, and which were not. Therefore, a multiple comparison procedure (post hoc testing) was also further adopted by employing the Tukey–Kramer range test in which \( p \) values < 0.05 were considered as significant. All data were represented as mean ± SD (standard deviation).

### 3. Results

The ultimate values of stress–stretch curves for spleen and liver tissues were evaluated from Equation (7), and are tabulated in Table 2 and depicted in Figure 2.

#### 3.1. Spleen

For the ultimate values in Table 2, there are at least two groups which are significantly different from each other \((p = 5 \cdot 10^{-7} \text{ for } \sigma_{ult}, \ p = 2.68 \cdot 10^{-38} < 0.05 \text{ for } \lambda_{ult})\). More specifically, post hoc Tukey–Kramer tests indicated that the frozen and cooled groups are significantly different from the fresh group \((p < 0.05)\). Remarkable decreases were identified in the magnitudes of the ultimate Cauchy stress and failure stretch for the cooled and freeze–thaw spleen compared to the fresh tissue. Nevertheless, no statistical variations were observed in the pairwise comparison between the cooled and freeze–thaw tissues \((p > 0.05)\).

Table 3 shows the constitutive parameters of the fresh, freeze–thaw and cooled spleen tissues. The fitted
curves were obtained with very good fit quality as depicted in Figure 5(a). A large difference can be observed for the average initial stiffness $k_0$ among all groups; the representative $k_{\text{fresh}}^{\text{0}}$ of the fresh group is not comparable with the others (see Table 3). The mean curves of the freeze–thaw and cooled tissues demonstrate remarkable shifts from the response of the fresh tissue, as plotted in Figure 3(b) and (c). Thus, the preservation conditions strongly affected the mechanical behaviour of the considered tissue resulting in stiffer spleen.

### 3.2. Liver

The failure values of the liver are plotted in Figure 2 and tabulated in Table 2. There were no statistically significant differences in the ultimate stress and failure stretch among the fresh, freeze–thaw and cooled liver tissues as indicated by ANOVA ($p = 0.719 > 0.05$ for $\sigma_{\text{ult}}$, $p = 0.069 > 0.05$ for $\lambda_{\text{ult}}$) and post hoc Tukey–Kramer tests.

The mean material coefficients obtained from curve fitting for the fresh, cooled and freeze–thaw liver are illustrated in Table 4. Obviously, there were no large differences in the average material constants as well as the mean initial stiffness coefficients for the three groups. Consequently, the stress–stretch curves for the frozen and cooled liver were unaffected by the preservation techniques. The average curves and the elliptical variation corridors of the liver tests are very similar as shown in Figure 4. The fitted curves in Figure 5(b) show almost the same behaviour through the full deformation range.

### 4. Discussion

#### 4.1. Effects of the freeze–thaw cycle on mechanical behaviour

Throughout the repeated tests with the identical methodology, the results agree with the hypothesis that decomposition occurs from autolysis in thawed organs (Nguyễn et al. 2012). In the freeze process, ice crystals might be unable to permanently destroy or break the elastin network, which contributes to the initial slope of the stress–stretch curve (Chow & Zhang 2011). Thus, it is likely that the thawing process may decrease the amount of cross-linking within the elastin network, leading to faster elastin decomposition. In our previous work (Nguyễn et al. 2012), strong decomposition occurred in elastin-rich spleen which tended to shift the material curve of the spleen close to the curve of the liver (capsule with parenchyma) by increasing the initial stiffness (see Figure 3). This increase by the freeze–thaw process correspondingly also reduced the maximum stretch of the splenic capsule (decreased by 59% in the ultimate strain). On the contrary, the liver was not affected by the freezing and thawing process. It is obvious that there was no liver decomposition as it does not contain elastin. Although employing a different experimental setup, our
findings on the liver capsule with parenchyma in uniaxial tension tests are consistent with the ones by Tamura et al. (2002). Freezing increased the stiffness of the porcine liver but decreased the spleen’s stiffness in indentation tests (Lu & Untaroiu 2012). These are contradictory to the findings in tensile tests by Nguyễn et al. (2012) in which the thawing process accounted for significant elastin decomposition of the splenic capsule since it is composed of tough fibrous layer, enriched with collagen and elastin. However, these contradictory results can be explained by the different loading. Thus, it remains crucially important to investigate the effects of preservation techniques in different experimental setups.

4.2. Effects of refrigerated storage on mechanical behaviour
Refrigerated storage strongly affected the mechanical properties of the porcine spleen by decreasing the failure stress and stretch values and increasing the initial stiffness, resulting in a stiffer tissue, see Figure 2 (decreased by 24% in the ultimate stretch or 57% in ultimate engineering strain). Nonetheless, the mechanical responses of liver tissues were almost unchanged. Despite using different preservation methods, our results seem to be comparable to the findings by Tamura et al. (2002) which showed no observable differences between thawed and fresh liver. Cold storage of bovine arteries at +4 °C significantly diminished the initial slope of the stress–strain curve in biaxial setup (Chow & Zhang 2011). However, no comprehensive reasons for the changes of the initial stiffness were indicated, the effects were supposed to be due to the changes in concentration and amounts of cross-linking within elastin network. Moreover, elastin mainly contributes to the toe region of the stress–stretch curve; therefore, elastin reduction will increase initial stiffness. This discrepancy might be due to the different cooling protocol (submerged in a different solution at different time points) in which the cross-linking within collagen network would be disrupted. Changes of the ultimate stress and of the Young’s modulus of refrigerated porcine arterial tissues at +4 °C after 24 and 48 h were found, but no differences are observed between the frozen (−20 °C or −80 °C) and fresh samples (Stemper et al. 2007). These findings are consistent with ours for porcine abdominal organs. The suggested effects were due to faster tissue degeneration during the refrigerated storage and thawing process. Preservation at +4 °C in EuroCollins solution for 21 and 31 days did not significantly affect human descending thoracic aorta in tensile loading; even a greater high strain modulus was noted without any explanation. However, the arterial allograft was found to be stiffer as preservation time increases (Adham et al. 1996). The cooling technique (+4 °C) resulted in a significant decrease of the stiffness of the spleen elucidated by softening effects of red pulp layer and a remarkable increase in liver stiffness due to extracellular structure strengthening from cell dehydration in indentation tests of porcine tissue (Lu & Untaroiu 2012). Our results showed the opposite pattern and no significant differences in the mechanical behaviour between the freeze–thaw and the refrigerated liver. Refrigerated storage (+4 °C) from 1 to 28 days resulted in changes in stress and stretch of mouse carotid artery for different durations of storage, caused by residual stress (Amin et al. 2011). However, when the elastin within arteries undergoes degeneration from storage conditions mechanical changes are observed.

Obviously, collagen contributes mainly to the stiffness in the quasi-linear region of the nonlinear stress–stretch curve while elastin contributes mainly to the low-stiffness toe region, see Figure 6 (Roach & Burton 1957). Our observations for the spleen after cooling preservation are similar to the trend in the stress–stretch curve over the hoop stretch in arteries for degenerated elastin or the trend in the stress–stretch curve of the spleen after the freeze–thaw cycle (Nguyễn et al. 2012). Thus, the digestion of elastin increases the
stiffness in the toe region and the material curve is then mainly characterized by collagen fibres. This finding demonstrates that decomposition of elastin occurs in the spleen during refrigerated storage as collagen decomposes at a slower rate. Similar trends were also observed in uniaxial tests (Nguyen et al. 2012) and in biaxial tests (Gundiah et al. 2013). In contrast to the observed changes in the spleen, no changes are seen for the liver. While splenic capsules have higher elastin content, liver contains a large amount of collagen but almost no elastin (Neuman & Logan 1950). Our results can also be quantified by the ratio of the average initial slope between the refrigerated and the fresh splenic capsules, which changed by a factor of six \( \left( \frac{k_{\text{cooled}}}{k_{\text{fresh}}} \approx 6 \right) \). In contrast, these values are close to one \( \left( \approx 1 \right) \) for the cooled and the freeze–thaw liver (see Tables 3 and 4). These results are consistent with our previous testing of freeze–thaw tissues (Nguyen et al. 2012).

Based on the average curves (Figure 5), our analyses suggest that the cooling storage and the freeze–thaw processes both had a negligible influence on the mechanical properties of liver specimens, but a significant effect on the spleen. Therefore, our main hypothesis is that not only the freeze–thaw cycle but also refrigerated storage do not directly result in micro-changes to the tissues but reduce the fraction of intact elastin fibres. This latter aspect is due to the acceleration of autolysis caused by the elastolytic enzymes in the cooling and thawing periods; the effects of both preservation techniques are nearly identical. Therefore, decomposition of non-degenerated proteins in the spleen shifts the curves in the direction of the values for livers (Figure 7). This trend was supported by our previous research (Nguyen et al. 2012) for the freeze–thaw cycle. Thus, it remains likely that a majority of decomposition occurs during the period of warming of the spleen up to RT. The preservation method could also affect the extra cellular matrix and a loss of smooth muscle cell viability in other tissues resulting in the decrease of ultimate strength (Venkatasubramanian et al. 2006). These storage techniques are intended to slow the tissue degeneration process, which may alter mechanics. However, our present study suggested that refrigerated storage as well as the freeze–thaw cycle should not be adopted for the spleen prior to biomechanical testing (Nguyen et al. 2012). Moreover, our statistical analysis showed that refrigerated storage results in identical effects on elastin-rich tissues (e.g. spleen) compared to the freeze–thaw cycle; no differences are found in the comparison between the cooled and the freeze–thaw liver tissue (Figures 3 and 4). After these preservation methods, all stress–stretch curves for both the spleen and liver tend to approach convergence (see Figure 7). It is clear that elastin decomposition could not contribute any mechanical properties to the overall curves in the physiological range. Therefore, all conclusions for the influence of the freeze–thaw cycle could be noted for the investigation of cooling effects. When the elastin cross-link network is decoupled by autolysis after tissue preservation, this decreases the failure stretch and hence increases the initial stiffness contributed mainly by collagen fibres. Consequently, elastin-rich tissue should be tested as freshest state possible; however, the mechanical properties of other tissues may not be compromised.

### 4.3. Limitations of the study

In this work, there are no separated tests for porcine liver parenchyma and liver capsule as well as for parenchyma/capsule spleen due to difficulty in removing parenchyma in spleen. However, the study by Kemper et al. (2010) can be considered for comparison, in which the mechanical behaviour of human liver parenchyma was quantified in tensile tests. The liver was obtained within 36 h and tested within 48 h of death. Between the time of procurement and specimen preparation, the tissue was immersed in a culture medium (Dulbecco’s Modified Eagle Medium) and chilled with wet ice. The comparison between porcine liver of the current study and human liver parenchyma by Kemper et al. (2010) at a strain rate 0.089 s\(^{-1}\) shows that the failure strain values are similar \( (\lambda_{\text{ult}} = 1.27 \) for porcine and \( \lambda_{\text{ult}} = 1.28 \) for human), whereas the failure stress value of porcine liver is significantly larger than the one of human liver \( (\sigma_{\text{ult}} = 184 \text{ MPa} \) for porcine and \( \sigma_{\text{ult}} = 77 \text{ MPa} \) for human), see Figure 2. That would be expected since our porcine liver specimens contain both capsule and parenchyma. Liver capsule composed of mostly collagen is stronger than liver parenchyma (Kemper et al. 2010). Moreover, Kemper et al. (2010) explained that the significantly larger failure stress of porcine liver is caused by the difference in the structure of human and porcine liver parenchyma. For example, there are differences in delineation of adjacent hepatic lobes between two

### Table 3. Fitted material parameters for spleen tissues.

| Number of samples \( n \) | \( C \) (MPa) | \( A_{11} \) | \( A_{12} \) | \( k_0 \) |
|----------------------------|--------------|-------------|-------------|-------|
| Fresh, \( n = 49 \) | 0.280 | 0.512 | 0.512 | 0.287 |
| Freeze–thaw, \( n = 50 \) | 1.494 | 0.512 | 0.441 | 1.530 |
| Cooled, \( n = 46 \) | 1.798 | 0.512 | 0.470 | 1.841 |

### Table 4. Fitted material parameters for liver tissues.

| Number of samples \( n \) | \( C \) (MPa) | \( A_{11} \) | \( A_{12} \) | \( k_0 \) |
|----------------------------|--------------|-------------|-------------|-------|
| Fresh, \( n = 62 \) | 0.814 | 0.512 | 0.404 | 0.549 |
| Freeze–thaw, \( n = 63 \) | 0.536 | 0.512 | 0.330 | 0.834 |
| Cooled, \( n = 65 \) | 0.747 | 0.512 | 0.367 | 0.765 |
species; in porcine, there is a physical boundary between adjacent lobes, whereas lobes in human liver are separated. In addition, the significantly higher proportion of connective tissue in porcine liver could also account for a significant increase in failure stress. However, attention should be paid since our porcine specimens were extracted from young three-month-old pigs, while the human liver specimens of Kemper et al. (2010) were taken from donors whose average age is 70.3 years.

The mechanical properties of capsule with parenchyma of porcine spleen in the current study were

![Figure 4. Average curves and elliptical variation corridors of liver tests (capsule with parenchyma).](image)

![Figure 5. Fitted curves to the average data.](image)

![Figure 6. Role of elastin and collagen for stress stretch curve of arteries (Roach & Burton 1957).](image)

![Figure 7. Fitted stress stretch curves for the elastin-low tissues.](image)
compared with the capsule/parenchyma specimens of human spleen by Kemper et al. (2012), in which all donor spleens were collected within 24 h and tested within 48 h of death with the preservation was similarly as described by Kemper et al. (2010). Specifically, the failure stress and failure stretch of porcine spleen are significantly larger than those of human spleen, see Figure 2. These are also consistent with the comparison of human spleen by Kemper et al. (2012) and porcine spleen in the study by Uehara (1995). The differences in mechanical properties between human and porcine spleen can be explained by difference of procurement, testing timeline, temperature, the variation of cellular architecture or the higher proportion of collagen and smooth muscle present in porcine spleen (Kemper et al. 2012). In addition, the average age of the donors used by Kemper et al. (2012) is 74 ± 5.8 years, which is not comparable to young three-month-old pigs used in our experiments.

Porcine organs compared to human abdominal organs may be greater in strength and elasticity (Stingl et al. 2002; Kemper et al. 2010, 2012). However, mechanical properties of the porcine kidney capsule are well matched with the corresponding ones of human (Snedeker et al. 2005).

Injuries of soft tissues may change their mechanical properties. Through impact acceleration test Shafieian et al. (2009) proved that viscoelastic properties of brain tissue significantly change after traumatic axonal injury. Most of the porcine livers in our experiments have been used in prior blunt tests of Grottke et al. (2010). The blunt test made a localized injury on an area 5 × 3 cm of one liver lobe. Although only intact parts of the liver were used for the tensile test, mechanical properties of the liver may be affected by blood loss and trauma induced coagulopathy. In fact, the tensile results of porcine liver are not divergent because fresh and stored liver are all tested after the same blunt tests (Figure 4). Hence, the blunt test may not severely change mechanical results of porcine liver.

However, this study suffered from some limitations. First, these investigations are limited to uniaxial tests of porcine tissues using quasi-static states. Methodological differences between pig and human tissue should be appropriately identified when applying to car-crash study. Moreover, errors stemming from quasi-static tests must be elucidated in order to be utilized in dynamic tests. Scaling methods for porcine abdominal organs can be carefully employed to study responses in humans for impact loading analysis (Kent et al. 2006, 2009). Second, it was assumed that the stress–stretch state was homogeneous within the specimen. This might be achieved if the length–width ratio of tissue specimens was about five (Ciarella et al. 2009). However, it was not possible to achieve this shape of the specimen due to insufficient organ size.

Third, the fitted material parameters are not unique, however they would be certainly used for making comparison when the same initial values of the coefficients remained unchanged during the optimization of the fitting process. In addition, the spleen may show weak anisotropy; hence, anisotropic material models could be recommended instead of isotropic models.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**References**

Adham M, Gournier JP, Favre JP, De La Roche E, Ducerf C, Baulieux J, Barral X, Pouyet M. 1996. Mechanical characteristics of fresh and frozen human descending thoracic aorta. J Surg Res. 64:32–34.

Amin M, Kunkel AG, Le VP, Wagenseil JE. 2011. Effect of storage duration on the mechanical behavior of mouse carotid artery. J Biomech Eng. 133:71007.

Chow MJ, Zhang Y. 2011. Changes in the mechanical and biochemical properties of aortic tissue due to cold storage. J Surg Res. 171:434–442.

Ciarella P, Dario P, Tendick F, Micera S. 2009. Hyperelastic model of anisotropic fiber reinforcements within intestinal walls for applications in medical robotics. Int J Robot Res. 28:1279–1288.

Duong MT, Nguyen NH, Staat M. 2012. Finite element implementation of a 3D Fung-type model. In: Holzapfel GA, Ogden RW, editors. 8th European Solid Mechanics Conference (ESMC 2012); July 9–13; Graz, Austria.

Foster CD, Hardy WN, Yang KH, King AI, Hashimoto S. 2006. High-speed seabelt pretensioner loading of the abdomen. Stapp Car Crash J. 50:27–51.

Franklyn M, Fitzharris M, Fildes B, Frampton R, Morris A, Yang KH. 2002. Liver and spleen injuries in side impact: differences by side of the road driven. In: Proceedings of IRCOBI; September 18–20; Munich, Germany.

Grottke O, Braunschweig T, Philippen B, Gatzweiler KH, Gronloh N, Staat M, Rossaint R, Tolba R. 2010. A new model for blunt liver injuries in the swine. Eur Surg Res. 44:65–73.

Gundiah N, Babu AR, Pruitt LA. 2013. Effects of elastase and collagenase on the nonlinearity and anisotropy of porcine aorta. Physiol Meas. 34:1657–1673.
Ikeyama K, Sakai H, Omasa M, Hamakawa H, Nakamura T, Fujinaga T, Fukuse T, Wada H. 2006. Influence of inflated lung pressure on lung mechanical properties during cold storage in rats. Eur Surg Res. 38:48–53.

Ikeyama K, Sakai H, Omasa M, Nakamura T, Hamakawa H, Fujinaga T, Fukuse T, Wada H. 2006. Effects of cold preservation on the lung mechanical properties in rats. Eur Surg Res. 37:85–91.

Kemper AR, Santago AC, Stitzel JD, Sparks JL, Duma SM. 2010. Biomechanical response of human liver in tensile loading. Ann Adv Autom Med. 54:15–26.

Kemper AR, Santago AC, Stitzel JD, Sparks JL, Duma SM. 2012. Biomechanical response of human spleen in tensile loading. J Biomech. 45:348–355.

Kent R, Salzar R, Kerrigan J, Parent D, Lessley D, Sochor M. 2009. Pediatric thoracoabdominal biomechanics. Stapp Car Crash J. 53:1–30.

Kent R, Stacey S, Kindig M, Forman J, Woods W, Rouhana SW, Higuchi K, Tanji H, Lawrence SS, Arbo gast KB. 2006. Biomechanical response of the pediatric abdomen, part 1: development of an experimental model and quantification of structural response to dynamic belt loading. Stapp Car Crash J. 50:1–26.

Lessley D, Crandall JR, Shaw CG, Kent RW, Funk JR. 2004. A normalization technique for developing corridors from individual subject responses. In: Proceedings of the SAE 2004 World Congress & Exhibition; March 8-11; Detroit, MI, USA. Paper 2004-01-0288.

Lu YC, Kemper AR, Untaroiu CD. 2014. Effect of storage on tensile material properties of bovine liver. J Mech Behav Biomed Mater. 29:339–349.

Lu YC, Untaroiu CD. 2012. Freezing and decay effects on material properties of porcine kidney and liver. Biomed Sci Instrum. 48:275–281.

Neuman RE, Logan MA. 1990. The determination of collagen and elastin in tissues. J Biol Chem. 45:549–556.

Nguyen NH, Dung MT, Tran TN, Pham PT, Grottke O, Tolba R, Staat M. 2012. Influence of a freeze–thaw cycle on the stress–stretch curves of tissues of porcine abdominal organs. J Biomech. 45:2382–2386.

Nicolle S, Noguer L, Palierne JF. 2012. Shear mechanical properties of the spleen: experimental and analytical modelling. J Mech Behav Biomed Mater. 9:130–136.

Nicolle S, Palierne JF. 2010. Dehydration effect on the mechanical behaviour of biological soft tissues: observations on kidney tissues. J Mech Behav Biomed Mater. 3:630–635.

Roach MR, Burton AC. 1957. The reason for the shape of the distensibility curves of arteries. Biochem Cell Biol. 35:681–690.

Shafieian M, Darvish KK, Stone JR. 2009. Changes to the viscoelastic properties of brain tissue after traumatic axonal injury. J Biomech. 42:2136–2142.

Snedeker JG, Niederer P, Schmidlin FR. 2005. Strain-rate dependent material properties of the porcine and human kidney capsule. J Biomech. 38:1011–1021.

Stemper BD, Yoganandan N, Sinennelli TA, Baisden JL, Pintar FA. 2007. Mechanics of fresh, refrigerated, and frozen arterial tissue. J Surg Res. 139:236–242.

Stingl J, Báča V, Cech P, Kovanda J, Kovandová H, Mandys V, Rejmontová J, Sohma A. 2002. Morphology and some biomechanical properties of human liver and spleen. Surg Radiol Anat. 24:285–289.

Tamura A, Omori K, Miki, Lee JB, Yang KH, King AI. 2002. Mechanical characterization of porcine abdominal organs. Stapp Car Crash J. 46:55–69.

Terriferi R, Gennisson JL, Tanter M, Beillas P. 2013. Effects of storage temperature on the mechanical properties of porcine kidney estimated using shear wave elastography. J Mech Behav Biomed Mater. 28:86–93.

Uehara H. 1995. A study on the mechanical properties of the kidney, liver, and spleen, by means of tensile stress test with variable strain velocity. J Kyoto Prefect Univ Med. 104:439–451.

Untaroiu CD, Bose D, Lu YC, Riley P, Lessley D, Sochor M. 2012. Effect of seat belt pretensioners on human abdomen and thorax: biomechanical response and risk of injuries. J Trauma Acute Care Surg. 72:1304–1315.

Untaroiu CD, Lu YC. 2013. Material characterization of liver parenchyma using specimen-specific finite element models. J Mech Behav Biomed Mater. 26:11–22.

Untaroiu CD, Lu YC, Siripurapu SK, Kemper AR. 2015. Modeling the biomechanical and injury response of human liver parenchyma under tensile loading. J Mech Behav Biomed Mater. 41:280–291.

Venkataramanat RT, Grassl ED, Barocas VH, Lafontaine D, Bischof JC. 2006. Effects of freezing and cryopreservation on the mechanical properties of arteries. Ann Biomed Eng. 34:823–832.