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Electrical Conductivity Method to Determine Sexual Dimorphisms in Human Temporomandibular Disc Fixed Charge Density

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Abstract

To investigate potential mechanisms associated with the increased prevalence of temporomandibular joint disorders among women, the study objective was to determine sex-dependent and region-dependent differences in fixed charge density (FCD) using an electrical conductivity method. Seventeen temporomandibular joint (TMJ) discs were harvested from nine males (77±4 years) and eight females (86±4 years). Specimens were prepared from the anterior band, posterior band, intermediate zone, medial disc and lateral disc. FCD was determined using an electrical conductivity method, assessing differences among disc regions and between sexes. Statistical modeling showed significant effects for Donor Sex ($p=0.002$), with cross-region FCD for male discs 0.051 ± 0.018 mEq/g wet tissue and 0.043 ± 0.020 mEq/g wet tissue for female discs. FCD was significantly higher for male discs compared to female discs in the posterior band, with FCD 0.063 ± 0.015 mEq/g wet tissue for male discs and 0.032 ± 0.020 mEq/g wet tissue for female discs ($p=0.050$). These results indicate FCD contributes approximately 20% towards TMJ disc compressive modulus, through osmotic swelling pressure regulation. Additionally, FCD regulates critical extracellular ionic/osmotic and nutrient environments. Sexual dimorphisms in TMJ disc FCD, and resulting differences in extracellular ionic/osmotic and nutrient environments, could result in altered mechano-electro-chemical environments between males and females and requires further study.

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Conflict of Interest

None of the authors of this paper have a conflict of interest that might be construed as affecting the conduct or reporting of the work presented.

Keywords

temporomandibular joint (TMJ); extracellular matrix; glycosaminoglycan content; porosity; mechanobiology

INTRODUCTION

Over half the population over age 50 shows degenerative changes to their temporomandibular joints (TMJs), increasing to 85% by age 75¹². Degenerative changes to the TMJ disc have been reported in 15–30% of asymptomatic joints, and as much as 85% of temporomandibular joint disorder (TMJD) patients demonstrate unilateral or bilateral degenerative disc changes^{23, 28}. TMJDs predominately affect women, often with greater severity of symptoms¹⁷. Fixed charge density (FCD), a measure of tissue matrix charge, has been suggested to be critical to TMJ disc load support.

Cartilaginous tissues possess a highly charged matrix, associated with the carboxyl and sulfate groups on extracellular matrix glycosaminoglycans (GAGs) (Figure 1)^{7, 22}. FCD measures these negative charges in each tissue unit¹⁴, with traditional FCD measurements including the tracer cation method (radio-labeled ions), imaging techniques (e.g. MRI), and mechanical indentation testing^{2, 20, 22}. Recently, an electrical conductivity approach has proved to be a reliable FCD measurement in bovine intervertebral disc tissues and human cartilage endplate^{14, 35}. This two-point electrical conductivity method determines fixed charge density from measurements of electrical conductivity on tissue samples equilibrated at two concentrations of bathing solution. When using a KCl bathing solution and considering the intrinsic ion diffusivities of K^+ and Cl^- are similar due to similar Stokes' radii, tissue FCD can be determined from the electrical conductivity measurements¹⁴. In addition to being a reliable method to accurately determine tissue FCD, the electrical conductivity method is easy to perform without special equipment, and should prove advantageous to those interested in tissue matrix composition, osmotic pressure, and nutrient and ionic environments.

Tissue FCD is an important regulator of cation and anion concentrations, Donnan osmotic pressure and swelling, and streaming potential/current within the tissue^{4, 7, 8, 21}. Studies have shown marked effects of changes to the ionic/osmotic environment on isolated or *in situ* fibrocartilage cell metabolism³⁶. By regulating cartilaginous tissue water content through Donnan osmotic pressure, FCD also affects tissue mechanical (e.g. swelling pressure and compressive modulus) and nutrient transport properties (e.g. hydraulic permeability and solute diffusivity)^{9, 31, 37}. Through regulation of osmotic pressure, FCD has been reported to resist as much as 50% of joint loads in the articular cartilage of the knee³³, and could play a significant role in the TMJ disc.

FCD has been studied in other cartilaginous tissues including the spinal intervertebral disc and articular cartilages^{11, 13, 14, 19, 21, 22, 29, 32, 35}. However, FCD of the human TMJ disc has not been investigated, or correlated with GAG content and porosity. Therefore, the purpose of this study was to determine region-dependent and sex-dependent differences in FCD using a two-point electrical conductivity method¹⁴, as well as GAG content and porosity, for

the human TMJ disc. Given regional differences in morphology¹⁰ and biochemical composition reported for porcine TMJ discs⁶, we hypothesized human TMJ discs would show region dependent variations in FCD, associated with regional variations in GAG content and porosity. Given the increased prevalence of TMJDs among women, and sex-dependent differences in electrical conductivity³⁴, sexual dimorphisms in temporomandibular disc FCD were also hypothesized, which would result in an altered mechano-electro-chemical environment between males and females.

MATERIALS AND METHODS

Specimen Selection and Preparation

Human TMJ discs were harvested in the Medical University of South Carolina Gross Anatomy Laboratory under institutional approval. Donor tissues were fresh frozen, and never formalin fixed. After removal, TMJ discs were morphologically screened to select healthy discs, wrapped in cellophane and PBS with protease inhibitor soaked gauze, placed in specimen bags and frozen until use at -20°C . In total, seventeen morphologically normal discs of the right side TMJs were harvested from nine males (77 ± 4 years) and eight females (86 ± 4 years).

Test specimens were prepared from the anterior band ($n=12$; 7M, 5F), posterior band ($n=13$; 7M, 6F), intermediate zone ($n=11$; 6M, 5F), medial disc aspect ($n=15$; 9M, 6F) and lateral disc aspect ($n=13$; 8M, 5F) using a 5 mm trephine (Figure 2, Top Left and Bottom Left). TMJ discs were sampled as disc size allowed, with some discs unable to provide specimens from all regions. Samples were microtomed, removing the superficial tissue layer and ensuring flat, concentric surfaces required for conductivity measurements.

Electrical Conductivity Measurements and FCD Calculation

FCD was determined by a two-point electrical conductivity method^{14, 35}. Following preparation, TMJ articular disc specimens were immersed in 0.15 M KCl solution (isotonic) for 12 hours at 4°C while axially confined between two hydrophilic polyethylene porous platens (50–90 μm , Small Parts, Inc., Miami Lakes, FL) and radially confined within a 5 mm diameter acrylic chamber, minimizing swelling and proteoglycan leaching. After incubation, electrical conductivity was measured by a custom conductivity instrument (Figure 2, Right)³⁴. Using a four-wire method, the resistance across the TMJ disc specimen was measured at a low and constant current density of 0.015 mA/cm^2 (Keithley Sourcemeter Model 2400, Keithley Instruments, Inc., Cleveland, OH). Specimen electrical conductivity (χ) was calculated by:

$$\chi = \frac{h}{RA} \quad (1)$$

where R , h , and A are the measured resistance, height, and cross sectional area of the specimen, respectively. After isotonic electrical conductivity measurements, specimens were re-equilibrated in 0.03 M KCl solution (hypotonic) for 12 hours and electrical conductivity measurements were repeated. All conductivity measurements were performed at room

temperature. Following each conductivity experiment, specimen height was measured by current sensing micrometer, to determine no specimen swelling occurred. Specimen FCD (c^F) was determined from the measured electrical conductivities in isotonic (0.15 M KCl) and hypotonic (0.03 M KCl) conditions, calculated by³⁵:

$$c^F = 2\sqrt{\frac{\chi_{Iso}^2[(c_{Iso}^*)^2 - (c_{Hypo}^*)^2]}{\chi_{Iso}^2 - \chi_{Hypo}^2} - (c_{Iso}^*)^2} \quad (2)$$

where χ_i and c_i^* (i = Iso or Hypo) are the measured specimen electrical conductivities and concentrations of the KCl solution under isotonic and hypotonic conditions, respectively.

Porosity and GAG Content Measurements

TMJ articular disc porosity (water volume fraction) was determined using the buoyancy method³⁷. Immediately preceding electrical conductivity experiments, specimen weight was recorded in air (W_{wet}) and in phosphate buffered saline (W_{PBS}) using a density determination kit and analytical balance (Sartorius YDK01, Germany). Immediately following electrical conductivity measurements, specimens were lyophilized and specimen dry weight (W_{dry}) recorded. Porosity (ϕ^w) was calculated by:

$$\phi^w = \frac{W_{wet} - W_{dry}}{W_{wet} - W_{PBS}} \frac{\rho_{PBS}}{\rho_w} \quad (3)$$

where ρ_{PBS} and ρ_w are the mass densities of the PBS solution and water, respectively. Lyophilized tissues were then assayed for GAG content by Blyscan Glycosaminoglycan Assay kit (Biocolor Ltd., Newtonabbey, Northern Ireland), and expressed as a ratio of dry weight ($\mu\text{g}/\text{mg}$ dry weight) and wet weight ($\mu\text{g}/\text{mg}$ wet weight).

Statistical Analysis

Statistical modeling assessed differences in FCD, GAG content ($\mu\text{g}/\text{mg}$ dry weight and $\mu\text{g}/\text{mg}$ wet weight), and porosity among disc regions (anterior, posterior, intermediate, medial and lateral), and between sexes (male and female). A general multivariate linear mixed effects model for dependent variables FCD, GAG content ($\mu\text{g}/\text{mg}$ dry weight and $\mu\text{g}/\text{mg}$ wet weight), and Porosity, evaluated differences for fixed effects Region and Donor Sex, controlling for random effects by including covariates for Donor and Donor Age. Where significant differences by sex were determined, regional contrasts were evaluated by two-tailed homoscedastic t-test with Bonferroni adjustment for multiple comparisons. Significant differences were reported at $p < 0.05$. Correlations between FCD and GAG content ($\mu\text{g}/\text{mg}$ dry weight and $\mu\text{g}/\text{mg}$ wet weight), and FCD and porosity were determined by Pearson product-moment correlation. Analyses were performed in SPSS (IBM SPSS Statistics, Version 23.0, IBM Corp., Armonk, NY), with descriptive statistics reported as mean $\pm$ standard deviation (SD).

RESULTS

The final multivariate general linear mixed effects model dropped the covariate Donor Age, which did not contribute significantly. The final multivariate model showed a significant effect for Donor Sex ($p=0.007$), while Region ($p=0.812$) and the interaction between Region and Donor Sex ($p=0.392$) were not significant.

Fixed Charge Density

FCD was reported as milliequivalent moles (mEq) per gram wet tissue. Pair-wise contrasts evaluated by the multivariate linear mixed effects model found significant differences between male and female FCD for TMJ discs ($p=0.002$) (Figure 3). Cross-region FCD for male discs was 0.051 ± 0.018 mEq/g wet tissue, while cross-region FCD for female discs was 0.043 ± 0.020 mEq/g wet tissue. FCD was significantly higher for male discs compared to female discs in the posterior band, with FCD 0.063 ± 0.015 mEq/g wet tissue for male discs and 0.032 ± 0.020 mEq/g wet tissue for female discs ($p=0.050$). There were no significant differences in FCD between males and females in the anterior band, lateral disc aspect, intermediate zone, or medial disc aspect. In the anterior band of the disc, FCD was 0.054 ± 0.018 mEq/g wet tissue for male discs and 0.043 ± 0.027 mEq/g wet tissue for female discs. In the lateral aspect of the disc, FCD was 0.051 ± 0.014 mEq/g wet tissue for male discs and 0.050 ± 0.020 mEq/g wet tissue for female discs. In the intermediate zone of the disc, FCD was 0.038 ± 0.016 mEq/g wet tissue for male discs and 0.042 ± 0.015 mEq/g wet tissue for female discs. In the medial aspect of the disc, FCD was 0.048 ± 0.021 mEq/g wet tissue for male discs and 0.047 ± 0.018 mEq/g wet tissue for female discs.

GAG Content

There was no significant difference in GAG content, expressed as $\mu\text{g}/\text{mg}$ dry weight, between male and female TMJ discs ($p=0.671$). GAG content for all specimens was 15.7 ± 6.6 $\mu\text{g}/\text{mg}$ dry weight, with cross-region GAG content for male discs 16.3 ± 6.1 $\mu\text{g}/\text{mg}$ dry weight and for female discs 15.0 ± 7.1 $\mu\text{g}/\text{mg}$ dry weight (Figure 4, Left). For male and female TMJ discs, GAG content was 15.4 ± 7.0 $\mu\text{g}/\text{mg}$ dry weight in the anterior band, 15.7 ± 5.4 $\mu\text{g}/\text{mg}$ dry weight in the lateral aspect of the disc, 15.4 ± 7.9 $\mu\text{g}/\text{mg}$ dry weight in the intermediate zone, 16.0 ± 6.6 $\mu\text{g}/\text{mg}$ dry weight in the medial aspect of the disc, and 16.5 ± 6.8 $\mu\text{g}/\text{mg}$ dry weight in the posterior band.

There was no significant difference in GAG content, expressed as $\mu\text{g}/\text{mg}$ wet weight, between male and female TMJ discs ($p=0.801$). GAG content for all specimens was 3.52 ± 1.67 $\mu\text{g}/\text{mg}$ wet weight, with cross-region GAG content for male discs 3.52 ± 1.34 $\mu\text{g}/\text{mg}$ wet weight and for female discs 3.53 ± 2.03 $\mu\text{g}/\text{mg}$ wet weight (Figure 4, Right). For male and female TMJ discs, GAG content was 3.63 ± 2.28 $\mu\text{g}/\text{mg}$ wet weight in the anterior band, 3.39 ± 1.06 $\mu\text{g}/\text{mg}$ wet weight in the lateral aspect of the disc, 3.43 ± 1.98 $\mu\text{g}/\text{mg}$ wet weight in the intermediate zone, 3.79 ± 1.83 $\mu\text{g}/\text{mg}$ wet weight in the medial aspect of the disc, and 3.48 ± 1.29 $\mu\text{g}/\text{mg}$ wet weight in the posterior band.

Porosity

There was no significant difference in porosity between male and female TMJ discs ($p=0.433$). Porosity for all specimens was 0.832 ± 0.033 , with cross-region porosity for male discs 0.833 ± 0.030 and for female discs 0.831 ± 0.037 (Figure 5). For male and female TMJ discs, porosity was 0.830 ± 0.040 in the anterior band, 0.841 ± 0.033 in the lateral aspect of the disc, 0.834 ± 0.035 in the intermediate zone, 0.831 ± 0.031 in the medial aspect of the disc, and 0.825 ± 0.028 in the posterior band.

Linear Correlation

Correlations between FCD and GAG content ($\mu\text{g}/\text{mg}$ dry weight and $\mu\text{g}/\text{mg}$ wet weight), and FCD and porosity were determined by Pearson product-moment correlation. There was a weak but significant correlation between FCD and porosity, $R^2 = 0.094$, $p=0.014$. There was no correlation between FCD and GAG content (% dry weight), $R^2 = 0.004$, $p=0.624$, or between FCD and GAG content (% wet weight), $R^2 = 0.025$, $p=0.225$.

DISCUSSION

This study used an electrical conductivity method to determine baseline, sex-specific FCD in human TMJ articular discs, and correlated results with GAG content and tissue porosity. Human TMJ articular discs showed no regional variations in FCD, GAG content or porosity, despite hypothesized variations based upon disc regional morphological differences. Although FCD of the human TMJ articular disc was smaller compared to other cartilaginous tissues, it plays a significant role in compressive load support. FCD was significantly higher in male discs compared to female discs, with significant differences in FCD between males and females in the posterior band of the TMJ disc. Sex-dependent differences in FCD in the human TMJ disc result in altered ionic/osmotic and nutrient environments between males and females, and could result in altered mechano-electro-chemical environments.

For the human TMJ disc, FCD was 4–5 times smaller than other cartilaginous tissues (Table 1). Based on the Donnan equation, electroneutrality condition and van't Hoff's equation for tissue osmotic swelling pressure⁸, cross-region FCD generates 11 kPa of osmotic pressure in males and 6.8 kPa of osmotic pressure in females. With the reported compressive modulus of the human TMJ disc in the range of 22–70 kPa with an average of 51 kPa¹⁵, osmotic pressure is approximately 20% of the overall compressive modulus of the TMJ disc. In comparison, the average FCD in healthy human knee articular cartilage (0.2 mEq/g wet tissue) can generate 155 kPa osmotic pressure, which has an average compressive modulus of 700 kPa²⁵. Thus, while the human TMJ disc has a smaller FCD compared to human knee articular cartilage, the relative contributions of osmotic swelling pressure to tissue compressive modulus are similar, contributing approximately 20% to the overall modulus for both the human TMJ disc and human knee cartilage. As such, the loading environment of the TMJ disc is not only defined by biphasic solid-fluid interactions^{19, 24}, but must be described by triphasic theory, considering the disc tissue as an ion exchange medium in which fixed negative charges within the tissue matrix resist fluid exudation and support loading through osmotic swelling pressure^{16, 18, 30}. These results confirm FCD plays a

significant role in compressive load support in the TMJ disc, and could result in an altered loading environment between males and females.

In addition to the contribution to TMJ disc biomechanical properties, FCD regulates the unique extracellular physicochemical environment inside the TMJ disc, elevating cation and reducing anion concentrations, and regulating extracellular osmotic pressure. Changes to the ionic/osmotic environment have shown significant effects on the behavior of intervertebral disc annulus fibrosus and nucleus pulposus cells and articular cartilage cells, such as energy metabolism and cell volume regulation^{1, 27}. Similar to intervertebral disc and articular cartilage cells, it is expected that maintaining the unique ionic/osmotic environment is critical for TMJ disc cell homeostasis. Under pathological conditions (i.e. degeneration), the FCD of other cartilaginous tissues decreases significantly³¹. It should also be considered, decreases in FCD due to proteoglycan depletion may reduce osmotic/swelling pressure, decreasing tissue dynamic compressive moduli and shifting tissue mechano-electro-chemical properties from equilibrium, which has been shown in intervertebral disc tissues³⁷. Subsequently, changes to FCD as a result of degeneration could be an important indicator of TMJ disc degeneration.

The greatest difference in FCD between male and female TMJ discs occurred in the posterior band, and while not significant, the greatest differences between male and female discs for GAG content and porosity also occurred in the posterior band. The posterior band of the TMJ disc could be particularly sensitive to differences in tissue mechano-electro-chemical properties. During jaw opening, the disc is squeezed forward over the condylar head, thus condyle-disc contact moves towards the TMJ disc posterior band. Compared to males, the female TMJ disc has significantly lower FCD in the posterior band, resulting in a smaller contribution of osmotic swelling pressure to the overall compressive modulus of the TMJ disc, with FCD generating 15 kPa of osmotic pressure in males and 3.9 kPa of osmotic pressure in females. In addition, compressive forces result in alterations to the ionic and nutrient environments within the TMJ disc due to tissue deformation, with mechanical strain having a greater impact on ion/nutrient diffusion in female discs compared to males³⁴. Changes to disc nutrient levels have been shown to profoundly impact TMJ disc cell proliferation and differentiation³. Overall, sex-specific differences in FCD result in different ionic/osmotic and nutrient environments in the TMJ disc between males and females, and could result in altered mechano-electro-chemical environments between males and females.

In the present study, there was no correlation between FCD and GAG content, likely attributed to the low GAG content in the TMJ disc, which is approximately 1.5% of dry weight. The measurement of FCD from tissue electrical conductivity has been shown to be a simple, robust and sensitive method^{14, 35}, compared to traditional tracer cation methods, magnetic resonance imaging and mechanical indentation^{2, 20, 22}. Other studies have established linear relationships between FCD and GAG contents^{14, 35}, likely due to differences in tissue type, which may contain higher GAG content. Regional variations in GAG composition have been reported for porcine TMJ articular discs, with the most abundant GAG types being chondroitin sulfate, dermatan sulfate and keratan sulfate⁶. Variations in FCD could be impacted by GAG composition, given differences in net charge between GAG types, where chondroitin sulfate carries 1.1–2.3 charged groups per

disaccharide, dermatan sulfate carries 2.0–2.2 charged groups per disaccharide, and keratan sulfate carries 0.9–1.8 charged groups per disaccharide⁴. While unable to be determined by this study, differences in GAG composition between males and females could in part determine reported differences in FCD.

In the present study, porosity (water volume fraction) was consistent with previously reported values for human TMJ discs, and similar to other fibrocartilages. In the present study, porosity for all specimens was 0.83, while previously reported mean porosity values for the temporomandibular articular disc was 0.80¹⁵. Across similar fibrocartilaginous tissues reported in the literature, typical tissue porosity ranged only from 0.80 – 0.86 (Table 1). Regional differences in tissue porosity have been reported. While not confirmed in the present study, tissue porosity has been shown to be significantly higher in the temporomandibular disc posterior band¹⁵.

Limitations with the present study include the large age range of the TMJ discs (71 – 91 years), with significant differences in age between male (77 ± 4 years) and female (86 ± 4 years) donors ($p < 0.001$). Donor age was not a significant study covariate ($p = 0.273$) however, and was removed from the final statistical model. Attempts were made to age-match male and female donors, however finding female discs with no signs of degeneration in the available sample population was problematic. More than twice as many female discs were sampled compared to male discs in this study. It should be considered that reported differences in FCD could result from proteoglycan loss due to early, non-observable degeneration. However, with no significant difference in GAG content reported between TMJ discs for males and females, it is anticipated that selected discs (morphologically normal) were generally healthy. Differences in FCD between males and females for this study population may be intrinsic, however further study is required. Ideally, this study would have included human TMJ discs with an age range relevant to the development of TMJDs, which typically occur in a younger population (20–40 years).

In conclusion, this study used an electrical conductivity method to determine baseline FCD for human TMJ articular discs, and established sex-specific differences in FCD between male and female discs. FCD was significantly lower in the posterior band of female discs compared to male discs. Although FCD in the human TMJ disc was smaller compared to other fibrocartilages, it plays a significant role in TMJ disc mechanical function. In addition, FCD regulates the TMJ disc unique extracellular ionic/osmotic and nutrient environment, critical for TMJ disc cell homeostasis. These sex-specific differences in FCD between male and female TMJ discs, and the subsequent extracellular ionic/osmotic and nutrient environments, could result in an altered mechano-electro-chemical environment between males and females.

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Figure 1.

Illustration of the collagen extracellular matrix demonstrating fixed negative charges, which attract mobile free ions, with glycosaminoglycans making up proteoglycans attached to hyaluronic acid cores. Fixed charge density measures the negative charges associated with carboxyl and sulfate groups on extracellular matrix glycosaminoglycans. Mobile ions (e.g., Na^+) neutralize the matrix fixed negative charges.

Figure 2.

Intact human temporomandibular joints were dissected from males and females, and articular discs were isolated (Top Left). Cylindrical specimens were taken from each disc from five physiologic regions: the anterior band, lateral disc aspect, intermediate zone, medial disc aspect, and posterior band (Bottom Left). Human discs were sectioned as size allowed; not all discs were able to provide all five specimens. Fixed charge density was determined using a custom electrical conductivity apparatus, consisting of two stainless steel current electrodes, two Ag/AgCL voltage sensing electrodes, a non-conductive Plexiglass chamber, a current-sensing micrometer, and sourcemeter (Right).

Figure 3.

Fixed charge density of human temporomandibular articular discs at each region reported for males and females. A significant difference ($p=0.050$) between males and females was determined in FCD at the posterior band of the articular disc.

Figure 4.
GAG content of human temporomandibular articular discs at each region for males and females reported as both % dry weight (Left) and % wet weight (Right).

Figure 5.
Porosity (water volume fraction) of human temporomandibular articular discs at each region averaged for males and females.

Table 1

Biochemical properties (Fixed Charge Density, GAG Content and Porosity) and Compressive Modulus for the temporomandibular joint articular disc, and selected cartilaginous tissues.

Tissue Source	Fixed Charge Density (mEq/g wet tissue)	GAG Content (µg/mg wet tissue)	Porosity	Compressive Modulus (kPa)
Temporomandibular joint disc (Present study)	Human: 0.05	Human: 3.5	Human: 0.83 Human ¹⁵ : 0.80	Human ¹⁵ : 51
Temporomandibular condylar cartilage	Porcine ¹⁹ : 0.04	----	----	Porcine ¹⁹ : 62
Intervertebral disc annulus fibrosus	Bovine ¹⁴ : 0.06 Human ³² : 0.13	Bovine ¹⁴ : 10 Human ²⁹ : 73	Bovine ¹⁴ : 0.81 Human ¹³ : 0.80	Bovine ²⁶ : 740 Human ⁵ : 30
Intervertebral disc nucleus pulposus	Bovine ¹⁴ : 0.19 Human ³² : 0.28	Bovine ¹⁴ : 34 Human ²⁹ : 103	Bovine ¹⁴ : 0.93 Human ¹³ : 0.86	Bovine ²⁶ : 310 Human ⁵ : 100
Human intervertebral disc cartilage endplate	Human ³⁵ : 0.12	Human ³⁵ : 31	Human ³⁵ : 0.73	Human ⁵ : 390
Articular cartilage	Human ²² : 0.12 Human ²¹ : ~0.20 Human ¹¹ : 0.02–0.15	Human ²⁵ : 50–100	Human ²⁵ : 68–85	Human ²⁵ : 700