Assessment of the correlation between GAGs and T_2 relaxation time using an MR imager.

Abstract

To determine a correlation between glycosaminoglycans and T_2 relaxation time by using magnetic resonance imaging. Gelatin hydrogels, of varying gelatin content, were created to study the affect of T_2. Chondroitin sulfate was added to the gels in increasing amounts to represent different levels of GAGs. The gels were tested in a GE Signa Excite 1.5 T MR scanner using a QID sequence developed at the University of Wisconsin. Analyzing the data revealed a negative correlation between the amount of GAGs in the sample to T_2 relaxation time. Further, we were able to demonstrate that a larger change in GAGs produced a greater change in T_2 value. The data did have many inconsistencies and the lack of correlation proves that testing must be normalized. This study provides evidence that a relationship between GAGs and T_2 can be obtained through the use of MR imaging techniques.

Key words: T_2; relaxation time; glycosaminoglycans; MR; degenerative disk disease.

Text

Introduction

Lower back pain has been responsible for much human suffering and increasing health costs. This type of pain is one of the most prevalent and costly to our society today. (1, 2) Much of this lower back pain is believed to be caused by intervertebral disk degeneration. In a healthy spine, the intervertebral disk is composed of a coarse fibrous ring, the annulus fibrosus (AF), which is surrounded by a central, translucent nucleus pulposus (NP). The main components of an intervertebral disk can be classified into four basic chemical groups: water, collagen, proteoglycans, and non-collagenous, non-proteoglycan proteins. Disk degeneration and aging is associated with changes in the concentrations of its chemical constituents (3-5). Water content is lost beginning from birth to death and more prominently in the NP than the AF. Further, there is more collagen in the AF than the NP and more proteoglycans in the NP that the AF. Both of these chemical constituents change with aging and degeneration, with collagen increasing and proteoglycans decreasing from their respective areas. (5)

MRI is a noninvasive clinical tool that has been used to document the state of an intervertebral disk. There have been many studies that have drawn relationships between different components of the intervertebral disk and relaxation values, T_1 and T_2 (6-10). Glycosaminoglycans (GAGs) make up proteoglycans and are of particular interest because they are known to be a significant factor in disk degeneration by holding in water and the loss of which contributes to disk degeneration (5). In the studies that have related T_1 to non-aqueous components of an intervertebral disk, it was found that T_1 is positively correlated to GAG concentrations and negatively correlated to collagen concentrations

(7). In other studies, T_2 and GAG concentration was found to be positively correlated (7, 6) while Weidenbaum et al. (11) found that there was no correlation between $1/T_2$ and GAG concentration. Overall, the literature that attempts to correlate relaxation times and chemical components of intervertebral disks is inconsistent and inconclusive.

With this problem on hand, Nightingale et al. performed a study to develop a model of the architecture of a human intervertebral disk based on the effect of the chemical components on its NMR properties (5). This study was successfully able to demonstrate that the solid components of the disk are responsible for most of the NMR properties and able to determine that as the disks degenerate, the same relationship between collagen and GAGs is maintained. This work serves to help increase specificity when applying MR techniques to studying disks in the future. These relationships between disk components and relaxation times were supported from another work by Antoniou et al. (12) that maintained the same T_1, T_2, water, GAG, and collagen relationships described by Nightingale et al. Unlike other efforts, in our study, we exclusively chose to examine the relationship between GAGs and changes in T_2, by utilizing hydrogels with varying GAG content. Collagen was not included in this study because its effect on T_2 is only significant at small T_2 values (5). In contrast to the Nightingale et al. study, we are analyzing our samples in the MR using a scan sequence that is common when assessing the health of a disk. This study looked at the correlation between proteoglycan content in a hydrogel to relaxation values (T_2) from MR images.

Methods and Materials

Gelatin samples

In order to obtain a homogeneous mixture of glycosaminoglycans and water, a matrix was needed to suspend the disk components in solution. Many gels were tested

including gelatin, agarose, acrylimide, and polyethyleneglycol (PEG), and gelatin was chosen to act as this matrix for a number of reasons. Not only is gelatin a widely available substance and easy to manipulate in the laboratory (13, 14), but upon testing in the MR scanner it had the lowest T_2 measurement making gelatin the most physiologically relevant.

Small, 10 mL glass vials were used to hold the gelatin samples. In order to obtain a range of T_2 values ideally between 50 and 150 ms, a range of gelatin samples were made between 9% and 23% gelatin (wt/wt %). After obtaining ideal T_2 values with plain gelatin samples, bulk solutions of each weight of gelatin (9, 13, 17, 20, 23 %) were made so that in future tests the samples were always from the same solution of gelatin. A preservative, sodium azide, was also added to help preserve the integrity of the gels over time. Having done this, glycosaminoglycans were added, specifically chondroitin sulfate A from bovine trachea cell. Because this specific chondroitin sulfate had a maximum solubility of 10%, small amounts of chondroitin sulfate were added such that gelatin samples were made with 0.2, 0.5, and 0.8 grams chondroitin sulfate. Table 1 shows the percent gelatin and GAG present in each sample made. The gel samples were refrigerated to ensure maximal stability and integrity.

Effect of position on samples

In order to assess the effect of position on the T_2 measurements, a set of gadolinium, or Gd, doped water samples was also developed. A relationship between the concentration of gadolinium and resulting T_2 value was found by using an NMR relaxometer. See Figure 1 for the relationship between gadolinium concentration and T_2 value.

Based on this relationship, a 90 mL stock solution was made with a T_2 equaling 80 ms. This was split between nine glass 10 mL vials so that there would be nine identical solutions for the measurements. These identical solutions were scanned in the MR to assess the scanner's accuracy and assess the effect of position on resulting T_2 measurement.

Phantom

A phantom was designed and constructed specifically for the needs of this project. Since the primary goal was to assess how glycosaminoglycans affect T_2 measurement, a phantom that eliminated the differences within the magnetic field was desired. The phantom was designed with the configuration and arrangement of samples in mind: the samples are parallel to one another such that only one plane of analysis is needed and also placed close enough to one another in order to minimize any variations in the magnetic field. With the phantom geometry chosen, one can place samples at distances ranging from 2.75 cm to 13.75 cm from the MR coil (spine, head) to assess this variation in space. The volume of the phantom was to be at least 2 liters to ensure adequate loading in the MR. Based on the dimensions, the phantom final volume was approximately 2.5 L. The diameter of the sample tubes is slightly larger than the vial diameter such that they are friction fit to eliminate most artifact from the material interface. The phantom was constructed at the Mechanical Engineering Lab out of acrylic and can be seen in Figure 2.

Pulse sequence and image analysis

A modified 3D fast spin echo (FSE) sequence was developed and used for the measurement of T_2. In phantom studies, the precision of the method was 2.4% for three separate trials (J. Perry, unpublished data). All data was collected on a GE Signa Excite

1.5 Tesla MR scanner. After obtaining the routine localizer image, the plane of image was selected such that the phantom's samples were sliced axially. The FSE sequence was modified in order to increase the precision and accuracy of T_2 calculation. This was done in three ways: the addition of a composite 180° refocusing pulses which minimize the effects of spatial heterogeneities, a series of crusher gradients with alternating sign and decreasing amplitude to rid the contribution from stimulated echoes, and the acquisition of all echoes at one phase encode value. The study used a 256 x 128 matrix, 24-cm field of view, 7.6-mm section, one average, echo train length of 32, T_E of 9.3 milliseconds, and T_R of 3 seconds, resulting in a 6:30 minute scan time (15).

The image analysis was performed by using a program written specifically for the purpose of determining T_2 measurements. The T2 value was calculated for each voxel by fitting the signal intensity for each T_E to both mono- and multi-exponential decay models by using a non-negative least-squares algorithm implemented in Matlab (MathWorks, Inc. Natick, Mass). The custom software preserved the spatial location of each voxel in the image by storing the data in matrices, which enabled us to create spatially accurate T_2 maps (15).

Results

In order to validate the consistency of the scans, both plain gelatin samples and gelatin samples with GAG added were scanned twice on two separate days. T_2 values were obtained for all the samples using a MATLAB program from an image similar to that shown in Figure 3. The magnitudes of these values obtained for the same samples increase significantly from Test Round 2 to Test Round 3. In most cases, the difference in magnitude of T_2 measurements between the two trials lies roughly within 100 ms and

200 ms. Despite the variation in magnitudes, the measured T_2 values obey similar trends; they can be affected by the concentration of GAG as well as the mass of gelatin. In fact, the presence of GAG can have significant effect on the T_2 values, especially in 1g and 1.5g gelatin samples, as indicated in Figures 4, 5, and 6. In general, GAG has a lowering effect on the T_2 values. When the mass of gelatin is kept constant, T_2 value will decrease as the concentration of GAG increases. In most samples, this observation is verified by the strong correlation R^2 value in Figures 4, 5, and 6. In addition, while the concentration of gelatin increases in the samples, the influence of GAG on T_2 values becomes less significant. This is demonstrated by the decreasing slopes of each group of constant gelatin mass, in the same scan, from lower to higher concentration of gelatin.

In Test Round 3, the samples having 0.5g of GAG were prepared several days before the other samples are made. Although the age of the samples is not the same, we still considered them as part of our data and used them to make further conclusions based on sample behavior. Nevertheless, the exclusion of these samples does not change the correlations between the GAG and T_2 values and between gelatin and T_2 values, as indicated by the similar R^2 values in Figures 6 and 7.

Each sample with GAG added was compared with its respective plain gelatin sample in terms of T_2 values. The difference of T_2 values between these samples were plotted in Figure 8. The variation of T_2 values increases as the decrease in GAG concentration becomes greater. This trend corresponds to the correlation of GAG and T_2 values deduced from Figures 4, 5, and 6. However, the dispersion of the data becomes greater for samples having higher concentration of GAG.

With the intention of verifying the consistency of the MR scanner, gadolinium samples having the same concentration were scanned seven times during two separate trials. These scans produced images similar to Figure 9. In most scans, the mean T_2 values were consistent, having magnitude roughly between 35 and 39 ms. However, the mean T_2 value of scan 1 of trial 1 was significantly lower than that of the other scans, indicating that this scan might be an outlier.

Discussion

Although the strength of the trend varied between the scan occasions, all of the T_2 measurements of the gelatin and glycosaminoglycan samples displayed the same correlation of GAG concentration to T_2 value (Figure 4 and 5). As small amounts of GAGs were added to a gelatin hydrogel, the average T_2 of the mixture decreased relative to the amount of GAGs added. This trend showed to be strong and consistent in several different concentrations of gelatin also. This observation is important for future studies attempting to model various soft tissue areas within the human body with the raw chemical components of which the tissue is composed.

Each scan also showed a consistent trend of a decrease in the magnitude of the slope of the GAG-T_2 relationship as the gelatin percentage increased. This is seen by comparing the linear fit equations of the gel percentages within one scan, Figure 4. Previous studies have shown T_2's strong dependence on the state and presence of water. Therefore, we hypothesize that in samples of lower gelatin concentration, the greater water percent interacting with the GAGs in the mixture causes a larger difference in T_2 with the same change in GAG percentage than in gelatin samples of higher

concentrations. This difference in T_2 change shows the possible interaction of GAGs with water when not arranged in a complex or matrix as they are in the body.

At this point, it is important to emphasize that the composition of the gel samples created in this study do not mimic the complex matrix of GAGs and collagen within the disks. Therefore, they do not display the same interaction between water and GAG as human intervertebral disks. Studies have shown that GAGs form a matrix that causes water to be retained within intervertebral disks (5). Therefore, because of the positive relationship between water content and T_2 value, a higher amount of GAG within a disk leads to a greater T_2 in healthier disks (5). This relationship is the reverse of the relationship we found between GAGs and T_2 within our gels and emphasizes the point that the gel samples created in this study are not a physiological model of GAG-water relationships within human intervertebral disks. This is even more evident because the glycosaminoglycans used in this study are solely chondroitin sulfate, even though human disks are a combination of several types of GAGs.

The large variability of the T_2 values between the scans taken at different times, Figure 4 and 7, presented an issue of inconsistency to our data. The degree of T_2 variation warrants discussion of its possible sources here. We do this in order to best define the variables that contribute to the correlation of this study's gel-GAG percentages and T_2 value. Although on average the T_2 values of Test Round 2 raised over 200%, the trends described between GAG and water, GAG and gelatin percent, and gelatin percent and T_2 change remained constant. The strength of the trends decreased with this increase of T_2 value. However, this variability and our limited resources have prevented us from drawing stronger conclusions from this data.

We looked to several factors for the cause of the dramatic increase in T_2 between our two test rounds. First, we considered the length of time the sample components had been mixed in the vial. We hypothesized that some kind of gelatin-GAG interaction over time could change the overall sample composition and therefore T_2 between test rounds. To test this we created Figures 4 and 6, the former being of a data set including data of the 0.5g GAG samples made at an earlier date than 0.2g and 0.8g GAG samples and the latter excluding those 0.5g GAG sample measurements. Because the inclusion of the 0.5g GAG samples did not significantly change the equation or R^2 value of the trend lines for each gelatin concentration, we can say that there was not significant interaction or change in gel properties that would affect T_2.

Another possible cause of the T_2 variability between test rounds is inconsistency within the MRI scanner. However, MR imaging has been found to be very accurate in several other studies, and not the cause of difference at the magnitude of differences found in our data (16). Secondly, two temporally different scans done of the phantom containing Gd samples proved that two similar results could be obtained in the MR we used by scanning the same object at different times.

Our third possible cause of the variability is the difference in the environment and condition the gels were scanned in between Test Round 2 and 3. Again, because of lack of resources, scans were not able to be carried out in the same manner each time. We discovered after our measurements were taken that the samples were scanned for Test Round 2 at a lower temperature (~10˚C) than in Test Round 3 (~25˚C). We hypothesize that this difference in temperature affected the movement characteristics of the water within our samples and therefore the T_2.

In Figure 8, the change in T_2 measurement - a result of the difference in GAG percentage between samples - is graphed against this GAG change. It represents the strength of trends that can be deduced from the data of this study. A linear fit is recognizable and may be helpful in future studies but not established enough to be a strong fit.

In conclusion, after completing the analysis of this study we were able to show a correlation between relaxation time (T_2) and GAG content in a gelatin hydrogel, by utilizing MR imaging. Although there are some inconsistencies in the data, we hypothesize that future analysis of such work could lead to a better fit for a graph that would be able to predict GAG change by observing a change in T_2. This study serves as a starting point in predicting a change in chemical composition of a disk by detecting the change in T_2. It is also a starting point for further analysis and verification with measurements from human cadaver intervertebral disks. By using the ideas presented in this study, we hope to emulate this scenario in cadaver disks by using MR imaging techniques and GAG digestion protocols. We expect that this work will help in the attempts to characterize the correlation between T_2 relaxation time and intervertebral disk composition.

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Tables

Table 1 (a) – Calculated gelatin weight per weight percent of array of 25 samples made for testing.

Gel Percentages - Weight/weight %					
		GAG Mass (g)			
Gel Mass (g)		**0.00**	**0.20**	**0.50**	**0.80**
	1	9.09	8.93	8.70	8.47
	1.5	13.04	12.82	12.50	12.20
	2	16.67	16.39	16.00	15.63
	2.5	20.00	19.69	19.23	18.80
	3	23.08	22.73	22.22	21.74

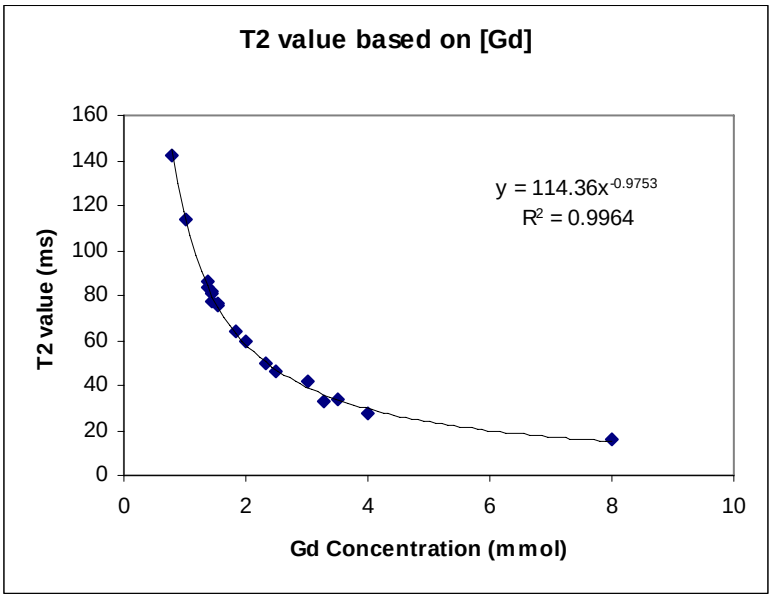
Table 1 (b) – Calculated chondroitin sulfate weight per weight percent of array of same 25 samples made for testing.

Chondrointin Sulfate Percentages - Weight/weight %					
		GAG Mass (g)			
Gel Mass (g)		**0**	**0.20**	**0.50**	**0.80**
	1	0.00	1.79	4.35	6.78
	1.5	0.00	1.71	4.17	6.50
	2	0.00	1.64	4.00	6.25
	2.5	0.00	1.57	3.85	6.02
	3	0.00	1.52	3.70	5.80

Figure Legends

1. The relationship between gadolinium concentration and resulting T2 measurement – As the concentration of gadolinium increases, the T2 value of the solution decreases. An equation was fit to the data for future gadolinium doped water sample calculations.
2. The 3-dimensional computer modeling of the phantom – The phantom was created with many specifications in mind, including distance between the sample and the MR coil, loading, and maximal elimination of artifact.
3. Scan image of samples for the 2nd scan of test round 3- In the center of the image, each circular shape represents a disk samples. The gradient of the circle reflects the composition of the sample. The ones having higher concentration of gelatin or GAG appear to be darker. For rows having 5 samples, the concentrations of gelatin are in the following order from left to right: 30%, 25%, 20%, 15%, and 10%. In the row having 4 samples, the order is 30%, 25%, 20%, and 15%, from left to right.
4. GAG concentration vs T_2 measurement – During the 2nd Scan of Test Round 2, 24 samples were scanned in the MR scanner and a strong relationship was revealed. This graph shows the inclusion of the 0.5g GAG data, which did not greatly alter the linear fit or R^2 value.
5. Consistency in the GAG concentration vs T_2 measurement relationship – During the 1st Scan of Test Round 2, 24 samples were scanned in the MR scanner. A strong relationship was revealed as well as consistency between scans when compared to the 2nd Scan of Test Round 2.
6. Consistency of GAG concentration vs T_2 relationship with differing sample age – Twenty four samples were scanned during the 1st Scan of Test Round 2 and the values of the 0.5g GAG samples are omitted in this figure to show the steady characteristics of the samples which determine T_2 over time.
7. Variability of GAG concentration vs T_2 relationship - In the 1st Scan of Test Round 3, 24 samples were scanned and much higher T_2 values were obtained as well as a weaker relationship between GAG concentration and T_2 value. The linear fits of this scan differ greatly from that Test Round 2.
8. Change in T_2 measurement due to change in chondroitin sulfate – The comparison of T_2 values belonging to samples containing differing amounts of chondroitin sulfate (weight per weight percentage) showed the trend of increasing T2 with decreasing chondroitin sulfate percent.
9. Scan image of phantom containing Gd samples- Gd samples having the same concentration were scanned to verify the consistency in the scans performed with the MR imager used in this study. The location of each Gd sample can be identified by each dark, round shape.

Figures



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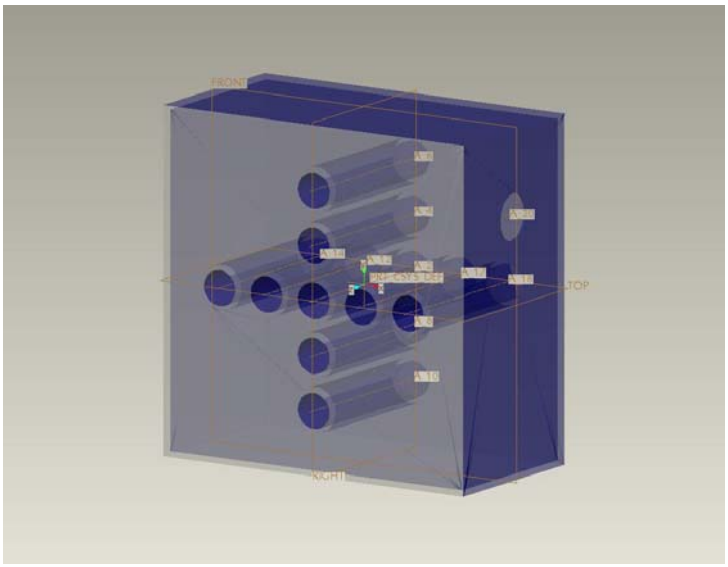

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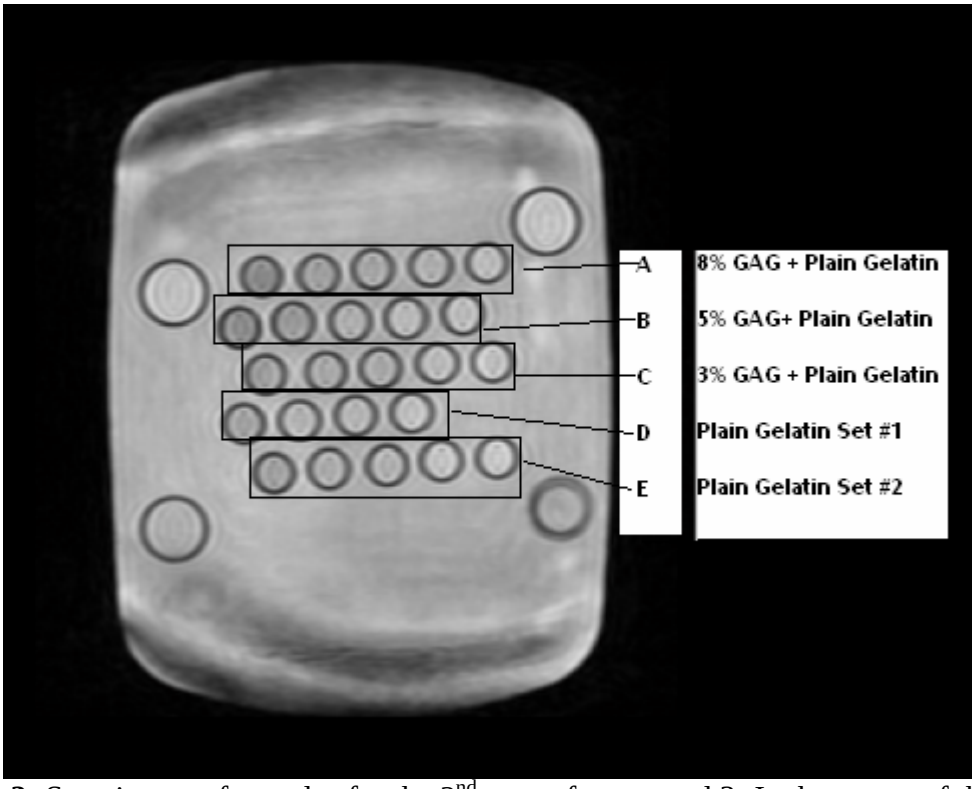

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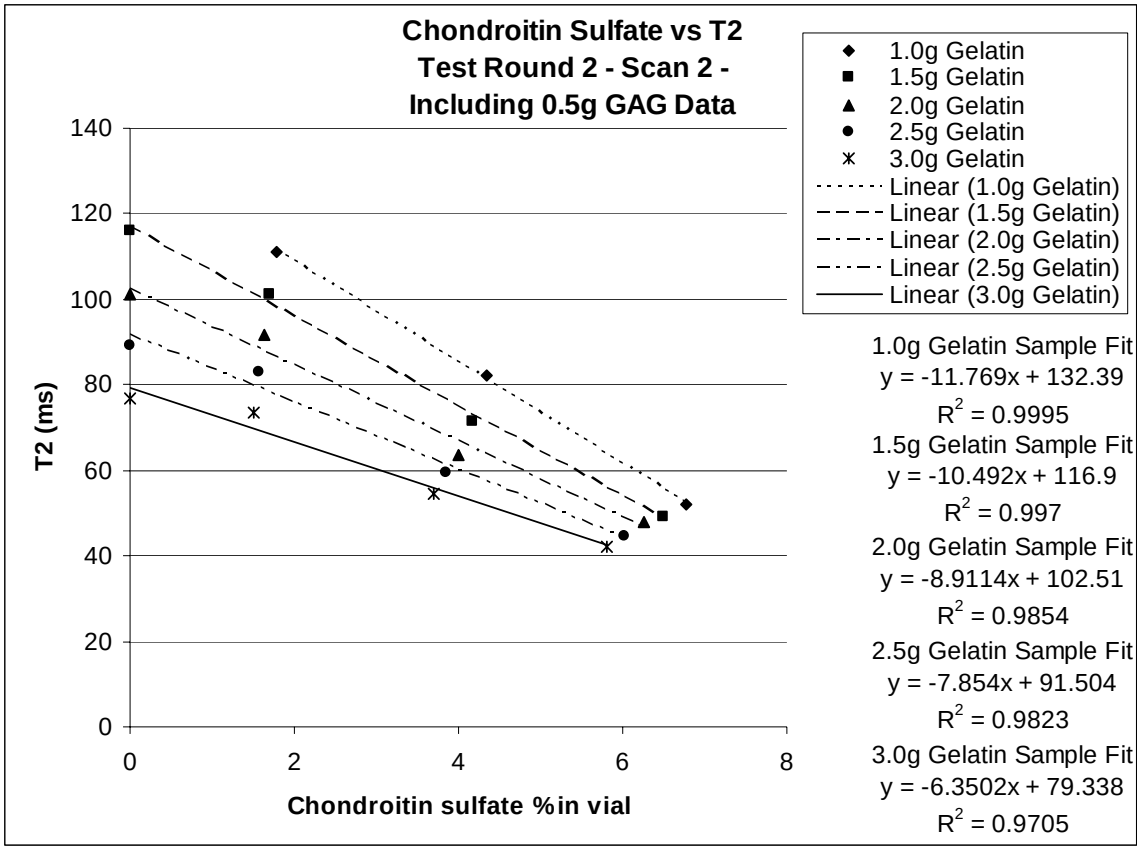

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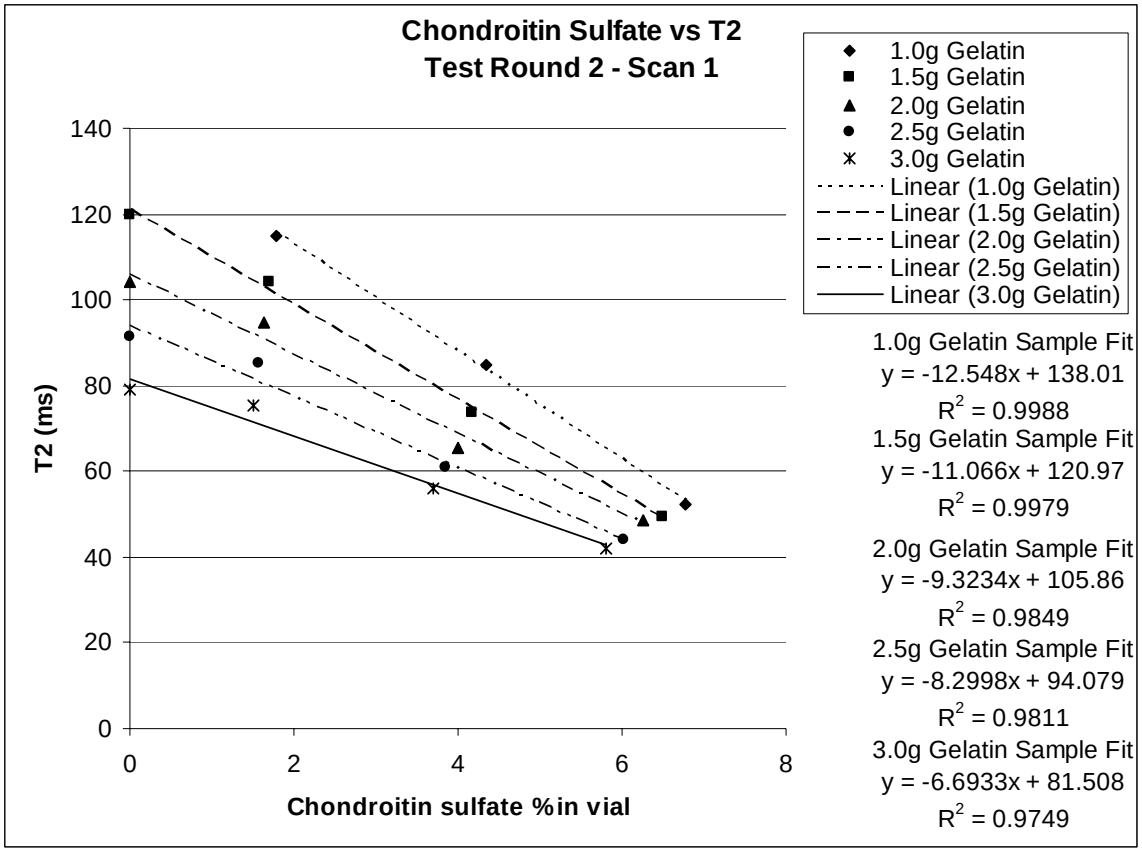

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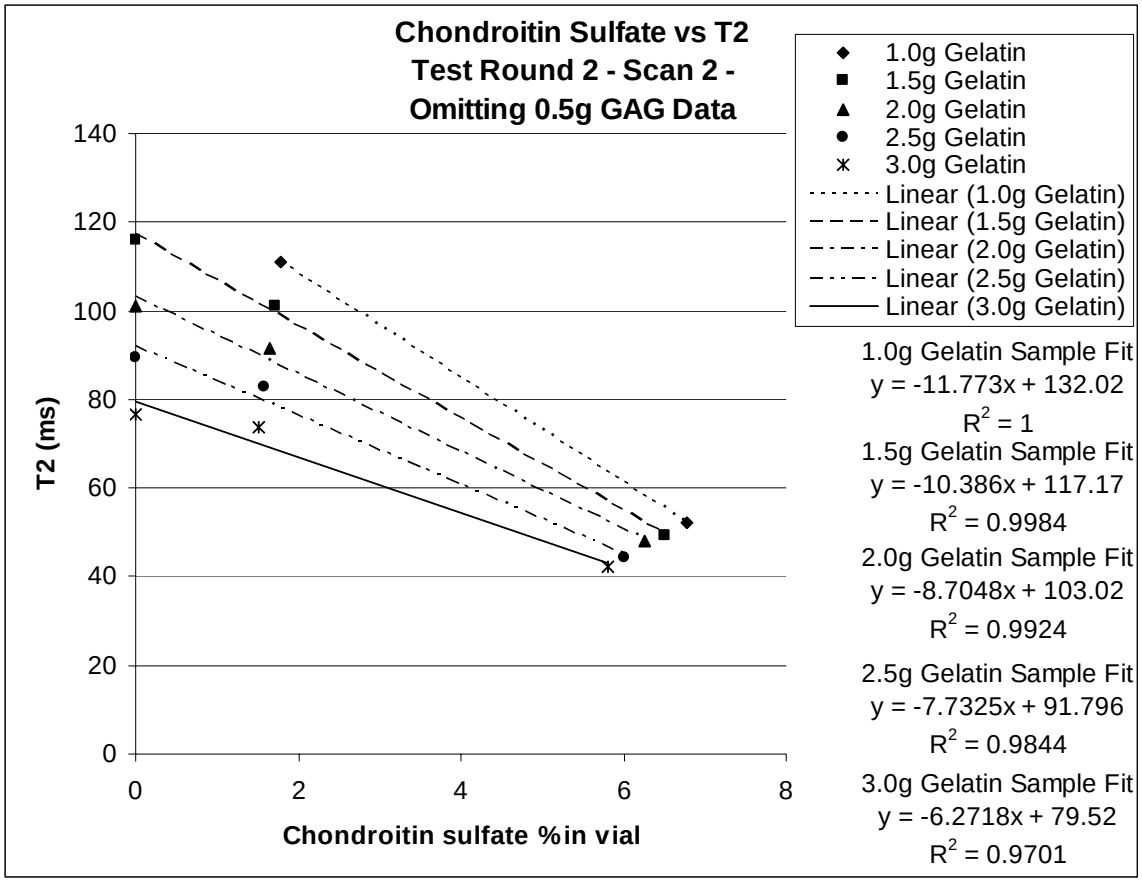

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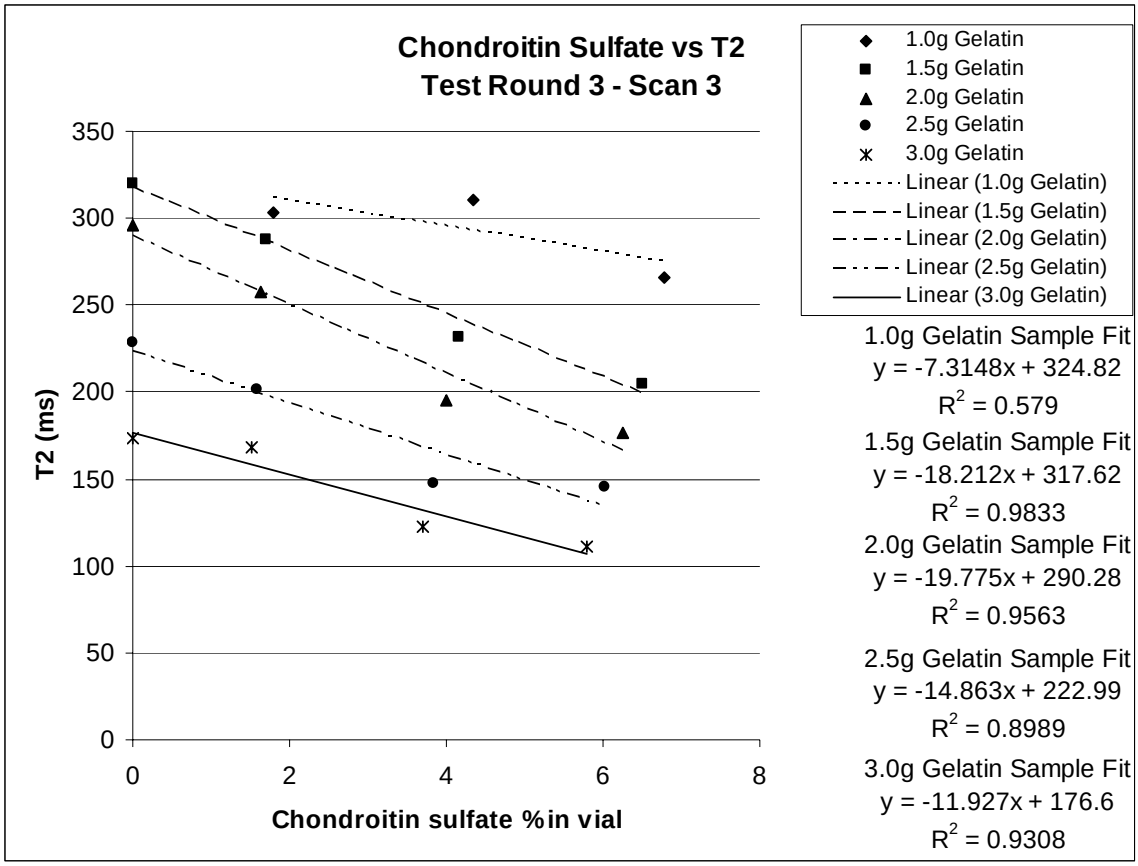

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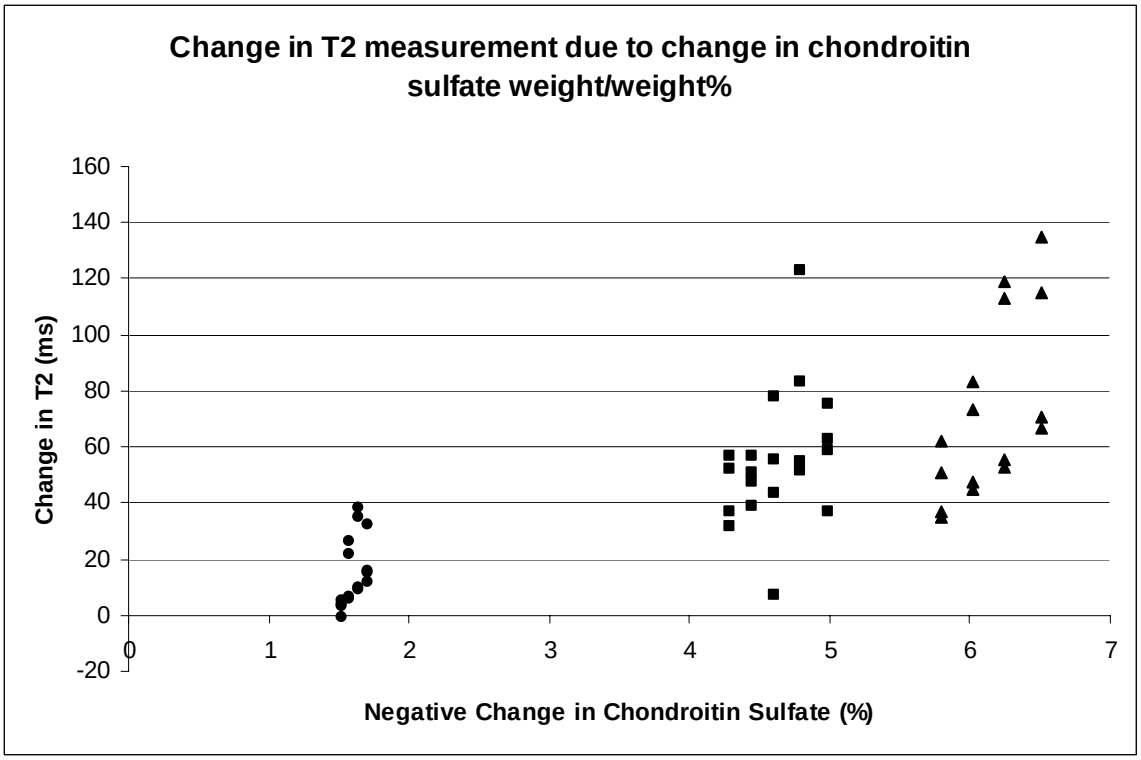

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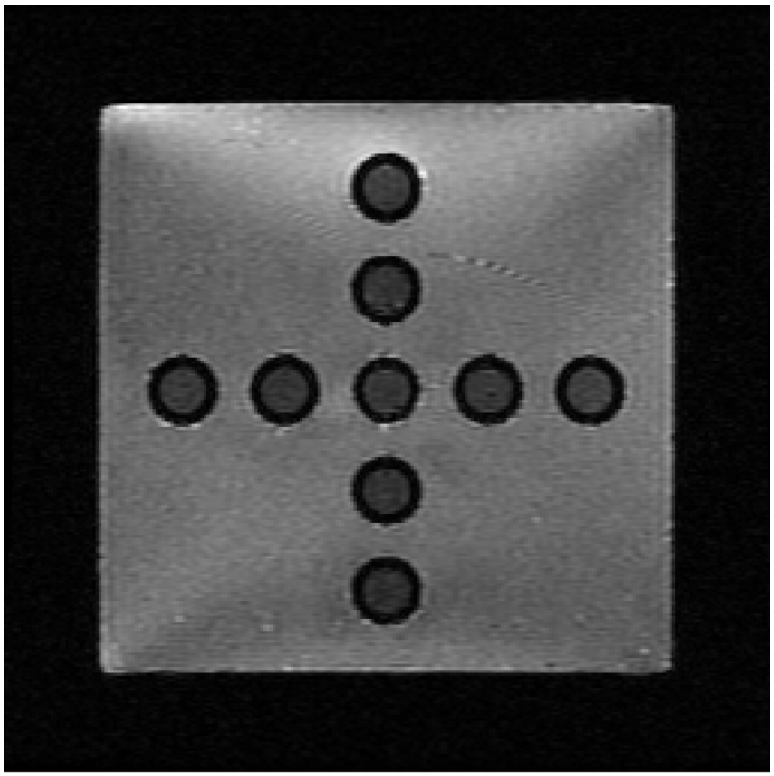

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