MRI measurement of Advanced Glycation End Products: The MRI hyperglycemic damage index.

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Introduction:

The number of diabetic patients in the United States has increased dramatically in the last decades. It has been established that diabetic complications are secondary to damage to bodily proteins from accumulation of Advanced Glycation End-products (AGEs) in poorly controlled patients. We are trying to develop an MRI measurement of collagen glycation in vivo that would be similar to the hemoglobin A1-C. That blood-test reflects control of diabetics for the previous three months – the approx. turnover time for red blood cells. Collagen is the most abundant protein in mammals; aging and hyperglycemia produce non-enzymatic cross-linking with AGEs (glycation) that accumulate over time given very slow turnover of the extra-cellular matrix. Such MRI method could reveal accumulated hyperglycemic damage over a longer time period. It could quantify accumulated damage to end organs in diabetics; it would help in the identification of diabetes treatments that will prevent complications, and could identify individuals with increased risk to develop those complications. Our hypothesis is that MRI can detect and measure glycation of tendon collagen in-vitro.

Methods:

Samples of fresh bovine digital flexor tendon were incubated in phosphate buffered saline (PBS) with 0.2 Molar glucose at 24ºC for up to 30 weeks. Control tendons were incubated in PBS without glucose. Sodium Azide 0.1 % was used as antimicrobial. A Philips Intera 1.5 T MR system was used with the Sense-Flex-S 11 cm dual surface coils. MRI of the samples was repeated at 1-3 week intervals using 3D T1 and T2 weighted gradient echo sequences as well as standard T1, PD, and T2 weighted spin echo sequences. Homogeneity correction algorithm (CLEAR) was applied. Scans were performed with samples parallel to Bo (P) and repeated with samples oriented at the Magic Angle (MA, 55º from Bo). Standardized ROI signal intensity (SI) measurements of tendons, incubation fluid, and background were recorded over time. Repeated measures ANOVA was used to test statistical significance of the SI difference between glycated and control tendons. The ratio of SI of samples at the MA versus parallel was used to develop a glycation index. The number of days incubated in 0.2 Molar glucose were converted to the equivalent in years at a concentration of 180 mg/dl (arbitrary level chosen to reflect uncontrolled hyperglycemia). For calculations we assumed a linear relationship between the concentration of glucose, the time of incubation and the amount of AGEs deposited in tissue.

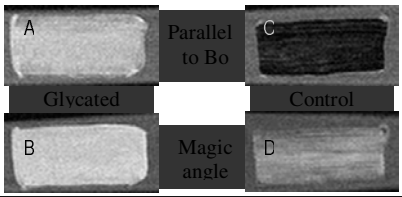

Figure 1: A). Sample glycated 100 days, parallel to Bo. B). Same glycated sample at the MA. C). Control tendon, parallel to Bo. D). Same control tendon at the MA.

Results:

Short TE sequences, especially the 3D T1 weighted spoiled-gradient echo (TR 25, TE 5, FA 20º), demonstrated the highest SI difference of glycated vs. control tendon and also the increased SI when positioned at the MA.

Large increase in SI over time was seen in glycated tendons but not in controls (See Figure 1). When imaged Parallel to Bo (P), SI increased up to 7.3 times over the controls. Repeated measures ANOVA demonstrated statistically significant difference ($p<0.001$). At the MA, SI of glycated tendons increased to a maximum of 1.5 times over the controls. ROI measurements of the control tendons, background and fluid showed minimal change of SI over time.

The glycation index (MA/P x 100) is depicted in Figure 2, It increases linearly up to 50 days of incubation in glucose (Equivalent to 2.5 years exposure to 180 mg/dl of glucose) with flat response thereafter that could reflect a binding saturation.

Conclusions:

MRI detects glycation of tendon in vitro as soon as 2-to-7 days (at least 8 weeks incubation in glucose has been needed by other authors to detect biochemical and biophysical change by High Performance Liquid Chromatography and by mechanical analysis).

The use of measurements at the MA to develop the index takes advantage of decreased difference between glycated and control samples at this orientation, this can minimize the need of an internal control when applied to diabetic patients in the future.

Strong dipolar interactions in normal tendon produce a very short T2 that is responsible for the very low SI in images parallel to Bo. The MA effect attenuates dipolar interactions of normal tendon and increases the T2, resulting in higher SI at the MA. The AGE cross-links disrupt the native collagen and produce attenuation of dipolar interactions, independent of the orientational effect, this results in increased SI both parallel and at the MA.

Sequences with shorter TEs and small flip angles are more sensitive to the effect of glycation since they are able to detect signal form substances with very short T2s.

Further studies are planned to validate results in diabetic patients.

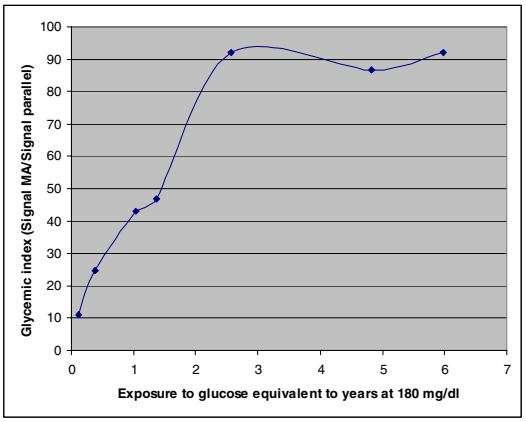

Figure 2: Glycation index ((SI at MA/Parallel) x 100) plotted versus time expressed as years of exposure to a glucose concentration of 180 mg/dl.