THE EFFECT OF NON-ENZYMATIC GLYCATION ON MECHANICAL PROPERTIES OF ARTICULAR CARTILAGE

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Introduction. During aging, many tissues are modified by non-enzymatic glycation (NEG) [1]. Sugars react with lysine residues in proteins to form crosslinks such as pentosidine [2]. Cartilage is particularly prone to NEG, due to the low turnover rate of collagen [3]. It has been previously shown that increased pentosidine levels can stiffen cartilage [2], and that threose, a sugar formed by ascorbate degradation [4], can affect the instantaneous deformation of cartilage [5]. It is possible that an increase in NEG crosslinks could stiffen the collagen network and predispose it to early fatigue failure, and hence, osteoarthritis. In addition, the modification of proteoglycan constituents could play an important role in tissue pathology. In this study the static and dynamic mechanical properties of bovine articular cartilage were measured after treatment with ribose and threose, and assessed to determine the effect of glycation on the poroelastic compressive and shear behavior of the tissue.

Methods. Intact 2-week old bovine calf knee joints were obtained from an abattoir. The joint was opened, and 9mm cartilage-bone cores drilled from the trochlear groove. Sterile PBS supplemented with antibiotics was used to keep the joints moist and cool during drilling. Three 1mm thick slices from each core were cut using a sledge microtome. Four plugs, 3mm in diameter, were punched from each slice. These plugs were then assigned to different groups, matching for position and depth on the joint surface. Plugs were wet weighed prior to incubation at 37^{O}C, 5% CO_2 for 5 days in PBS at pH 7.4 (with 25 mM EDTA and 0.02% Sodium Azide to limit cell mediated enzymatic degradation) in the presence of one of the following: Ribose (50, 200, or 500mM), Threose (200 or 50mM), or no sugar as a non-glycated control. At the end of the incubation, the plugs were placed in PBS alone to equilibrate for 1 hour, and their wet weight measured. The equilibrium and dynamic moduli in uniaxial unconfined compression and also in shear were found by testing three plugs at a time from a group in an incubator housed loading machine under axial or angular displacement control [6]. First the plugs were compressed by 10% to ensure full contact with all of the plugs. Then, to find the compressive equilibrium modulus, the plugs were compressed by 2% in 30s and allowed to stress-relax for 300s. This was repeated 2 more times to allow an equilibrium stress–strain graph to be plotted, the slope of which was taken to be the equilibrium compressive moduli of the three plugs. Dynamic axial moduli were calculated at 3 frequencies (1, 0.5, 0.1Hz) by imposing a 2% sinusoidal strain and calculating the resultant stress amplitude. The shear equilibrium modulus was calculated by imposing 3 consecutive 1% ramp and hold shear displacements, followed by stress relaxation. Shear dynamic moduli were found at a shear strain amplitude of 1% and frequencies of 1, 0.5, and 0.1Hz.

Results. The compressive and shear moduli of plugs incubated in all three concentrations of ribose were not significantly different from control plugs (figure 1). Threose however, caused a significant increase in all of the moduli at both 50 and 200mM (figure 1). The shear moduli of the 200mM threose-incubated plugs were also significantly greater than the 50mM group. Compressive moduli only showed a trend for this result. Over the 5 day incubation, the control plugs increased in wet weight by almost 30% (figure 2). Ribose-incubated groups only swelled by 20%, while threose treated plugs showed little or no weight increase (figure 3).

Discussion. Threose was shown to be a much more potent stiffening agent compared to ribose. While no difference was seen in the moduli for ribose as compared to control plugs, it clearly was having some effect on the matrix, as wet weight did not increase as much as in control values. This could indicate that either the effective crosslink density within the collagen network had increased, or the swelling pressure of the proteoglycans had decreased. This effect was even more pronounced in the threose treated plugs. It has been reported previously that in these short term, high concentration sugar incubations, the proteoglycans are glycated more than the collagen [7]. How glycated proteoglycans differ in mechanical properties is unknown. In previous studies [2,5], instantaneous deformation tests were used which highlight the role of the collagen network [8]. This study was designed to see if additional measurements of cartilage stiffness were also affected. The glycation of proteoglycans is most likely to affect the compressive equilibrium modulus and, to some extent, the shear behavior of cartilage [9], as measures of the balance between collagen network tension and proteoglycan charge repulsion. However, axial dynamic moduli will depend largely on fluid pressurization of the collagen network at high frequency, when little fluid flow occurs, but additionally on the proteoglycan component at lower frequencies. In the simple shear configuration used, there is little or no fluid flow, and there no volumetric change [6], so again, shear moduli depend largely on collagen network properties, but also reflect proteoglycan interactions [9]. Taken together, our data suggest that glycation has likely affected proteoglycan as well as collagen constituents in modifying the spectrum of biomechanical properties. Ongoing studies compare the quantity of glycation products accumulated in the tissue over the incubation period, as well as looking at the effect of incubating in ascorbate, the parent compound of threose. It is possible that an increased matrix stiffness due to accumulation of glycation end-products could make the collagen network prone to fatigue failure.

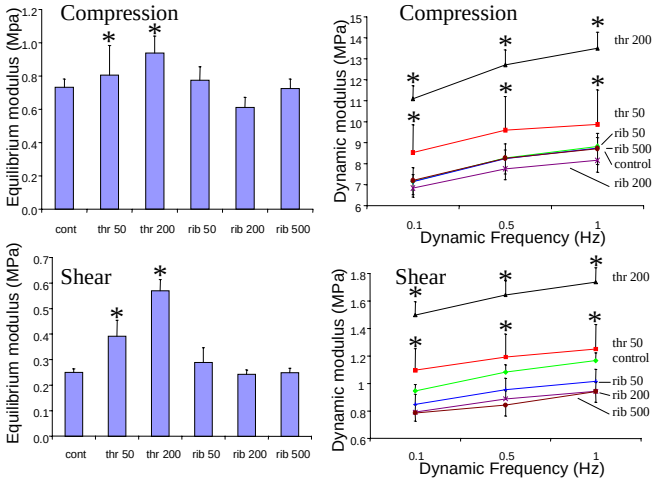

Figure 1. Compressive and shear equilibrium moduli (left) and dynamic moduli (right). (n=12, mean +sem, * = p < 0.05 vs. control modulus)

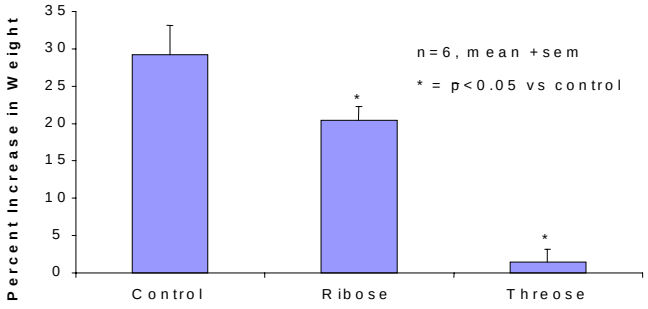

Figure 2. Wet weight increases over 5 day incubation period

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