Figure 5: Effect of cB on indentation relaxation function, R(t), for step 5 as a typical step, for controls (grey line) and cB specimens (dark line). Relaxation function at 24 hr is divided by relaxation function at 0 hr to give the curves for control and cB. Error bars represent the standard deviation near the end of the test for the specimens in that group. Left error bar is for the controls; right error bar is for the cB digested specimens. There was no statistically significant difference between control and cB for any of the steps.

Figure 6: Average relaxation function for the tension tests for the second step, R2(t), after digestion divided by R2(t) before digestion, for control specimens (CONS/S; N = 3) and specimens treated with cB (cBS,M/S,M; N = 7). There was no statistically significant difference from one in either average curve due to the incubation and CON is not different from cB at any time point.

Material to be included as Supplementary Material:

cB buffer: 50 mM trizma-base, 50 mM sodium acetate, 1 mM calcium chloride pH 8.0 containing protease inhibitors 5 mM NEM, 0.5 mM PMSF, 1 mg/ml Benzamidine and antibiotics at 50 μg/ml gentamicin)

Proteoglycan extraction and assay: Proteoglycans were removed from the tissue after all mechanical testing by moderate agitation in 10 volumes of 4 *M* guanidine chloride, 250 m*M* sodium acetate, 10 m*M* EDTA, 5 m*M* tryptamine-HCL, 2.5 m*M* phenanthroline, 10 m*M* NEM and 0.5 m*M* PMSF, pH 5.8, at 4°C for 24 hoursextraction [26]. The extracted proteoglycans were dialyzed three times in buffer containing 0.25 *M* sodium acetate, 0.02% sodium azide, pH = 7.0 and three times in dH_2O at 4°C and lyophilized to dryness. Proteoglycans were reconstituted in dH_2O and the protein content determined using the BioRad protein assay. For tensile specimens, tissue was weighed and then digested in 1 mg/ml proteinase K (Pierce Chemical) in 100 m*M* Na_2HPO_4, 50 m*M* EDTA, pH = 7.4 for 18hrs at 56° C and then the proteoglycan content determined and data reduced to mg of proteoglycan per ml of wet weight tissue.

Justification for method of data reduction of mechanical data: We chose to quantify our mechanical data by equilibrium and rapid modulus and relaxation curves, rather than a unified constitutive equation, such is often done in cartilage testing. We did this because of what we believe is controversy in choosing such equations, in particular whether to use a biphasic model or a poroviscoelastic model. The former accounts for water flow in cartilage response; the latter includes both the water flow and potential solid viscoelasticity. The difficulty is that we have found no method to separate the two effects. Huang[36] proposed use of a tension specimen to identify viscoelastic parameters and a compression test to identify poroelastic parameters, but this assumes the viscoelastic properties are the same in tension and compression. We tried this and were not able to fit our data. The problem appeared to be that the viscoelastic response was not the same in tension and compression. Rather than try to resolve this problem, we chose to

identify properties in the very fast (rapid modulus) and very slow (equilibrium modulus) regimes, states where most investigators agree that elastic properties can be assumed. We chose the relaxation curve as a measure of the time dependency, whether due to poroelasticity or viscoelasticity. We believe this is valid since we are only interested in change, not in absolute parameter values.