# NON-ENZYMATIC GLYCATION ALTERS THE CREEP AND VISCOPLASTIC PROPERTIES OF HUMAN CORTICAL BONE

**Deepak Vashishth, Ping Cheng Wu and Gary B. Gibson**

Department of Biomedical Engineering
Rensselaer Polytechnic Institute
Troy NY 12180

Bone and Joint Center
Henry Ford Hospital
Detroit MI 48202

## INTRODUCTION

Collagen is considered to be the principal contributor of post-yield properties. Any modification of bone collagen including accumulation of non-enzymatic cross-links should, therefore, be expected to modify the fracture behavior of bone. Previous studies under monotonic loading to fracture have shown that non-enzymatic glycation (NEG) of bone reduces its post-yield properties [1,2], but no information is available on the extent to which NEG may alter the damage behavior of bone. For example, it is unknown if NEG modified bone, containing some amount of damage, undergoes progressive or instantaneous fracture under load. Such information may be critical to the understanding and treatment of age-related fragility fractures because, with aging, bone accumulates NEG products [1] and microdamage [3].

Using stress relaxation tests on demineralized bone and multiple load cycle creep tests on mineralized bone, it is shown here that *in vitro* NEG of bone stiffened the collagen matrix and modified the creep and inelastic behavior associated with the formation and propagation of damage in bone. NEG-mediated modification of bone quality, therefore, results in an instantaneous and brittle fracture at physiological strain levels. Furthermore, NEG content, measured from demineralized bone, was also shown to predict the stiffness of collagen matrix and damage properties of mineralized bone.

## METHODS

Fourteen dumbbell specimens of 3 mm diameter were machined from bilateral human tibiae (Age/Sex: 46/F). The specimens were separated into two groups of 7 specimens each. The first group, designated as glycation group, was immersed in a 0.6 M ribose solution at 37°C for 7 days to induce cross-links by glycation. The other group, designated as control group, was immersed in the same solution for 7 days but without ribose. Glycation was confirmed by examining the change in specimen color (Fig. 1) and by measuring the fluorescence based NEG content. Previous studies suggest that the change in bone color to orange corresponds to the doubling of NEG content and that the doubling of in-vivo NEG content corresponds to 3 decades of aging [1,2].

After incubation, specimens in both groups were subjected to standard stress controlled creep tests incorporated into a multiple load cycle scheme under physiological conditions (37$^{o}$C saline). Each load cycle contained a one second ramp-up load, one-minute hold period under constant load, one-second ramp-down to zero load and one minute hold period at zero load. The above sequence was repeated several times on each specimen using a greater load on each cycle until failure. The initial load level (25 MPa) on each specimen was increased by 25 MPa for the next 2 cycles (50 MPa and 75 MPa) and then by 5 MPa (80 MPa, 85 MPa, etc.). An extensometer was used to measure the strain. Creep rates were calculated using strain and time data at each stress level. The maximum strain, creep rate and residual strain at each load level and initial stiffnesses were compared between the control and glycated groups.

Following the multiple load cycle creep tests, one 6 mm long cylindrical specimen was harvested from the grip-end of all tested specimens (control and glycated). Cylindrical specimens were then demineralized in EDTA solution and subjected to stress relaxation tests in order to determine the equilibrium modulus and diffusion characteristics of the organic matrix.

After testing, demineralized cylindrical specimens were papain digested (0.4 mg/ml papain in 0.1 M sodium acetate buffer, pH 6.0, 16 h, 65˚C) and used for measurement of NEG content. Non-enzymatic glycation content was determined from fluorescence using 370 nm excitation and 440 nm emission wavelength standardized against a quinine sulphate solution [1].

## RESULTS & DISCUSSION

Extent of Glycation: Consistent with the color change (Figure 1) seven days of in vitro ribosylation lead to the doubling of NEG content. Fluorescence based analysis indicated that NEG content in glycated specimens (640±22 ng fluorescence/mg collagen) was double and significantly higher than in control specimens (315±37 ng fluorescence/mg collagen) ($p<0.001$).

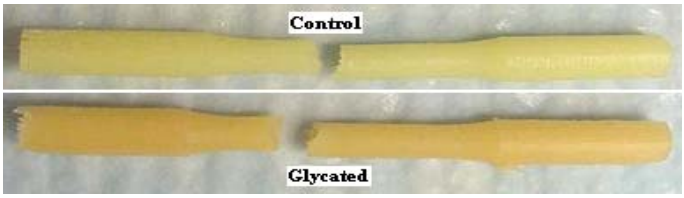


**Figure 1**: Test specimens. Glycation was confirmed by examining the specimen's change in color from white to orange and by measuring the fluorescence based NEG content.

Properties of mineralized bone and correlation with NEG content: Doubling of NEG content did not increase the stiffness of bone [Glycated (22±7 GPa); Control group (21±4 GPa); $p=0.95$] but altered its damage and creep behavior. At failure there was a 42% strain reduction in the glycated group (0.37% strain) compared to the control group (0.64% strain) (Fig.2 $p<0.03$). Significant differences in the steady creep behavior between the two groups were observed at all stress levels > 95 MPa. The control group's creep rate (34.59 μstrain/s) was 3 times or more than the glycated group (9.61 μstrain/s) for all stress levels > 110 MPa ($p<0.04$). The glycated group had significantly lower residual strain (700±169 μstrain) than the control group (1163±265 μstrain) for all stress levels > 105 MPa (40% or more reduction) ($p<0.01$).

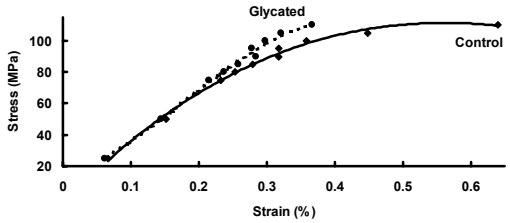


**Figure 2**: Stress vs. strain. Glycated bone failed at 42% lower strain than control. Note that higher strain rates used here reduced the strain to failure and the strain to failure in glycated bone is well within the physiological range reported in the in vivo strain gage studies.

The natural spread in the NEG content in the glycated and control groups allowed for the testing of correlation between NEG content and the creep and inelastic properties of the mineralized bone. NEG content showed a significant negative correlation with the creep and inelastic properties of mineralized bone ($p<0.05$) (Figure 3). NEG content, measured in patients from bone biopsies, may therefore be a significant predictor of the fracture properties of the mineralized bone.

Stiffness of the organic matrix and correlation with NEG content: Consistent with previous bovine bone data, equilibrium modulus was significantly higher ($p<0.01$) in the glycated (4.17±0.47 MPa) than in the control (2.35±0.97 MPa) group (p) but the diffusion characteristics were not different ($p=0.78$). Furthermore, the correlation test over the limited range of data, available from this study, showed a significant positive correlation between the NEG content and the equilibrium modulus of demineralized bone ($R^2=0.64$; $p<0.01$).

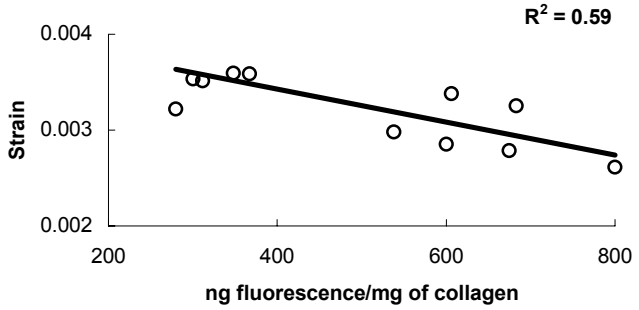


**Figure 3**: Maximum strain at a given level of stress is linearly and negatively correlated with the NEG content.

In summary, non-enzymatic glycation of bone, corresponding to three decades of aging, stiffened the organic matrix, reduced creep rates and residual strain, and caused bone to fracture instantaneously when loaded under impact to physiological levels of strains. Age-related accumulation of NEG content in bone is therefore likely to alter the organic matrix stiffness, reduce damage processes and contribute to bone fragility.

## REFERENCES

[1] Vashishth, D., Gibson, G.J., Khoury, J.I., Schaffler, M.B., Kimura, J., and Fyhrie, D.P. (2001). Influence of non-enzymatic glycation on biomechanical properties of cortical bone. *Bone*, 28, 195-201.

[2] Catanese, J., Bank, R.A., Tekoppele, J.M., and Keaveny, T.M. (1999). Increased cross-linking by non-enzymatic glycation reduces the ductility of bone and bone collagen. *Proceedings of the ASME 1999 Bioengineering Conference*, 42, 267-268.

[3] Schaffler, M.B., Choi, K., Milgrom, C. (1995). Aging and matrix microdamage accumulation in human compact bone. *Bone*, 17, 521-525.