Non-Enzymatic Glycation is Associated with Markers of Bone Quality in Human Cortical and Cancellous Bone

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INTRODUCTION:

Factors other than low bone mass, such as changes in bone quality, may contribute to fracture risk. Non-enzymatic glycation alters bone’s organic matrix [1] and creates collagen crosslinks, collectively termed advanced glycation end products (AGEs) [2]. AGEs accumulation with age stiffens bone's organic matrix, ultimately leading to fracture [3]. Cell culture work in animal models shows increased AGEs decrease osteoclastic activity and reduce bone turnover [4]. Through such alterations in resorption and turnover, non-enzymatic glycation can influence the bone microstructure as well as the matrix and influence bone fragility in both cortical and cancellous bone. Our goal was to investigate the influence of non-enzymatic glycation on bone’s microarchitecture, which in turn affects bone’s fracture resistance through the formation of specific morphologies of microdamage (linear microcracks versus diffuse damage). We hypothesized that trabecular rods will be more glycated than plates and that highly glycated regions of bone will produce more linear microcracks.

METHODS:

Human cancellous bone cores (ages 18-97) were mechanically tested via uniaxial compression using established protocols [5] and stained with lead-uranyl acetate to detect microdamage formation [6,7]. 22 trabeculae were extracted from male (n=7) and female (n=7) donors. They were imaged with micro-computed tomography (microCT) to determine SMI, where higher values indicate more trabecular rods and lower values indicate more trabecular plates. The ratio of damaged surface area to volume ratio (DS/DV) for microdamage was calculated in order to characterize microdamage morphology, where higher values indicate linear microcracks and lower values indicate diffuse damage. Trabeculae were then hydrolyzed and quantified for fluorescent AGEs with a fluorometric assay [8]. 9 human cortical bone beams were fatigue tested and stained with basic fuchsin to detect microdamage. They were sectioned into 4 µm thick transverse sections and mounted on specially designed membrane slides. Linear microcrack and diffuse damage regions were laser-microdissected and collected in isolation caps (Figure 1). An extraction buffer was added to each tube and incubated to extract proteins (24 hours, 4°C). Protein extracts were dialyzed against 1x PBS (24 hours, 4°C), and extracts were used for quantification of a specific AGEs, pentosidine, using recently developed methods [9].

Outliers were determined as any measurements beyond two standard deviations of the mean of the group and were removed from analysis. Correlations were run between SMI and DS/DV; and between SMI and fluorescent AGEs for trabeculae. T-tests were run between AGEs quantity in linear microcrack and diffuse damage regions.

RESULTS:

In trabecular bone, we found a positive correlation between SMI and DS/DV (r=0.48, p<0.05), indicating that trabecular rods produce crack-like microdamage (Figure 2). Furthermore, we found a positive correlation between SMI and total fluorescent AGEs (r=0.49, p<0.05), indicating that trabecular rods were also highly glycated (Figure 1). In cortical bone, we found that microdissected regions containing linear microcracks were more glycated, as indicated by pentosidine quantity normalized to collagen, than regions containing diffuse damage (LMC: 9.40 ± 4.97 nmol/µmol, DD: 4.45 ± 3.30 nmol/µmol, p=0.06).

DISCUSSION:

Our results indicate that changes in bone’s extracellular matrix by non-enzymatic glycation are responsible for the formation of specific morphologies of microdamage. This mechanism is similar in both cancellous and cortical bone where highly glycated regions of bone produce harmful microdamage that results in decreased fracture resistance.

In cancellous bone, less glycated trabecular plates undergo resorption on the surface to leave behind more glycated and weaker rods. In contrast to plates, increased non-enzymatic glycation in trabecular rods is associated with linear microcracks. The high glycation level and increased microcrack content weakens the cylindrical structures.

Similarly in cortical bone, we found that linear microcrack regions were more glycated than diffuse damage regions. Cortical bone is considered to have a lower overall metabolic rate compared to cancellous bone. The already low turnover combined with additional inhibition of resorption due to increased AGEs accounts for the accumulation of harmful microdamage. Thus, in both cortical and cancellous bone tissue, bone areas containing increased AGEs are weakened, and these regions have reduced ability to efficiently dissipate energy due to the formation of linear microcracks.

SIGNIFICANCE:

The significance of this work lies in demonstrating that non-enzymatic glycation affects the transition from plate-like to rod-like trabecular structure and microdamage morphology.

Figure 1. Laser capture microdissection was used to cut regions of microdamage. Images show a linear microcrack before cutting (left) and after removal from the bone slice into an isolation cap (right).

Figure 2. (Left) Trabecular rods accumulate more crack-like damage while plates accumulate more diffuse-like damage. (Right) Trabecular rods are more glycated than plates.

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