Effects of β-aminopropionitrile and Exercise on Type I Collagen Morphology in Murine Bone

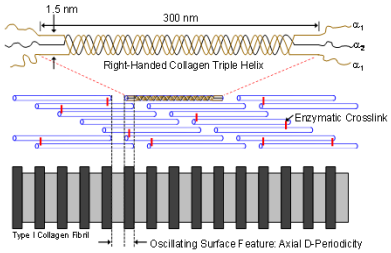
Max A. Hammond[1], Joseph M. Wallace[1,2]

[1]Purdue University, Weldon School of Biomedical Engineering, West Lafayette, IN

[2]Indiana University-Purdue University at Indianapolis, Department of Biomedical Engineering, Indianapolis, IN

INTRODUCTION

Type I Collagen



Osteolathryism

- Lysyl oxidase (LOX) catalyzes crosslink formation stabilizing collagen fibril
- β-aminopropionitrile (BAPN) toxin blocks LOX active site reducing crosslinking
- Well understood mechanism allowing investigation of role of enzymatic crosslinks in collagen morphology and bone mechanics

Exercise

- Loading increases size and changes bone physicochemical properties
- Post-yield improvements and altered gene expression implicate changes in collagen

Study Contribution

- Detect morphological changes in collagen with reduced crosslinks, exercise, and affects of disease in the presence of exercise
- If bone mechanical integrity is rescued, exercise could be a potent non-invasive treatment for collagen-based diseases.

HYPOTHESIS

Disease-induced alterations to collagen's nanoscale morphology can be compensated for via exercise

MATERIALS AND METHODS

Animals

- 8 wk old female C57BL/6 mice
- Cage activity (Sed) or exercise (Ex)
  - 30 min/day, 12 m/min, 5° incline, 21 consecutive days
- 200 µl subcutaneous injection
  - PBS or 300 mg/kg BAPN in PBS
- Sacrificed at 11 weeks (n=4-5 per group)
- Femora and tibiae harvested, stored at -20°C

AFM Imaging and Analysis

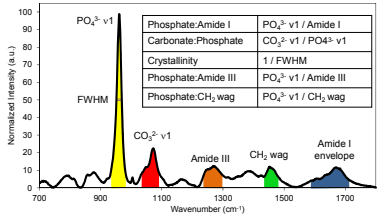
- Left femur polished, demineralization with EDTA, 4-5 locations per bone
- 3.5 µm x 3.5 µm images in air
- D-periodic spacing from 2D Fast Fourier Transform (2D FFT) power spectrum
- 15-20 fibrils/location, ~70 total/sample

Nanoindentation

- Right femur polished, 5 locations per bone, hydrated sample
- Diamond Berkovich indenter
  - 300 µN/s to 3000 µN, held for 10 sec, and unloaded at 300 µN/s
- Reduced modulus (E_r) and hardness (H) using Oliver-Pharr method

Raman Spectroscopy

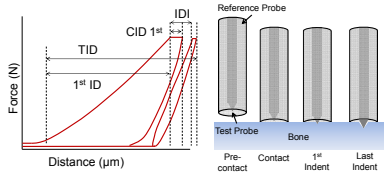
- Left tibiae, 5 locations per bone
- Distal to tibia-fibula junction
- Unprocessed hydrated surface
- Gaussian fit for phosphate peak FWHM



- Band area ratios calculated, linear baseline

Reference Point Indentation (RPI)

- Distal tibia used after Raman
- Sample hydrated in PBS bath
- 2 N indents for 10 cycles, BP3 probe
- Cycle by cycle analysis using MATLAB script



Statistical Analysis

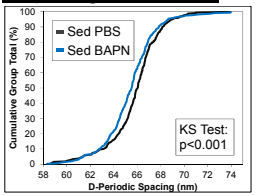
- Mean comparisons using two-way ANOVA
  - Ex and BAPN main effects
  - Transformation if assumptions violated
- D-spacing distribution differences tested with Kolmogorov-Smirnov (KS) tests
  - Bonferroni correction for multiple comparisons, p<0.0167 significant

RESULTS

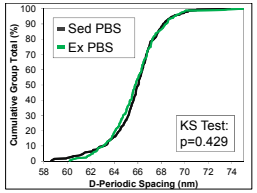
Group	RPI: 1st ID (µm)	RPI: IDI (µm)	Raman: Crystallinity *	Nanoindentation: E_r (GPa)	Nanoindentation: H (GPa)
Sed PBS	35.38 ± 3.87	6.50 ± 0.50	0.0535 ± 0.0003	12.9 ± 1.7	0.63 ± 0.12
Sed BAPN	35.03 ± 1.38	7.51 ± 1.15	0.0537 ± 0.0004	11.4 ± 2.5	0.55 ± 0.16
Ex PBS	40.09 ± 4.83	7.73 ± 2.72	0.0532 ± 0.0004	11.9 ± 3.7	0.58 ± 0.23
Ex BAPN	36.38 ± 4.40	6.89 ± 2.05	0.0540 ± 0.0004	14.2 ± 2.3	0.74 ± 0.19

* p<0.05 for BAPN, † p<0.05 for Ex, ‡ p<0.05 for interaction

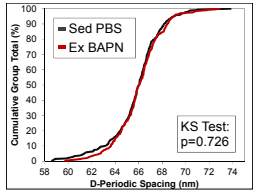
D-spacing Distribution



BAPN alters distribution



Ex does not alter distribution

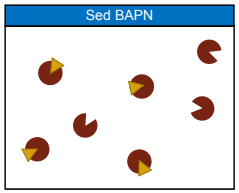


Exercise with BAPN returns distribution to control

DISCUSSION

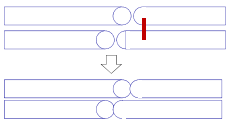
Mean D-spacing

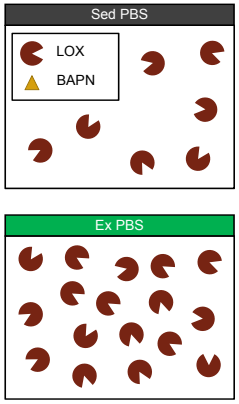
- D-spacing exists as a distribution, mean only describes small part
- Sample size limits power



Distribution Changes with BAPN

- BAPN blocks some LOX active sites reducing functional LOX
- Reduced enzymatic crosslinks result
- D-spacing distribution shifts lower possibly due to the compression of spaces normally occupied by crosslinks
- Downward shift may explain increased crystallinity



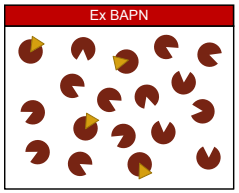


Distribution Does Not Change with Ex

- Increased production of enzymes necessary for bone formation (such as LOX) in response to load
- Enzymatic crosslinks are tightly regulated and only occur at specific locations
- Increase in overall LOX with exercise does not alter distribution because number of crosslinking sites within a given fibril does not change

Control Distribution

- LOX initiates crosslinks only at specific sites of the collagen fibril in a regulated fashion



Distribution Rescued by Ex from BAPN

- BAPN still blocks some LOX
- Overall LOX increased due to exercise
- Normal level of BAPN-free functional LOX resulting in normal crosslinking

No Change in Mechanics

- Sample size limits power
- 1st ID trend with Ex possibly due to periosteal growth

Limitations and Future Directions

- AFM captures young tissue
- Increase sample size for mechanical analysis
- qPCR to verify increase in LOX production

Exercise in the presence of BAPN treatment restores the collagen D-spacing distribution to normal in murine bone.