

# Nanoscale Compositional and Mechanical Alterations of Bone Tissue at Peri-Lacunar Regions in Type-1 Diabetic Postmenopausal Women



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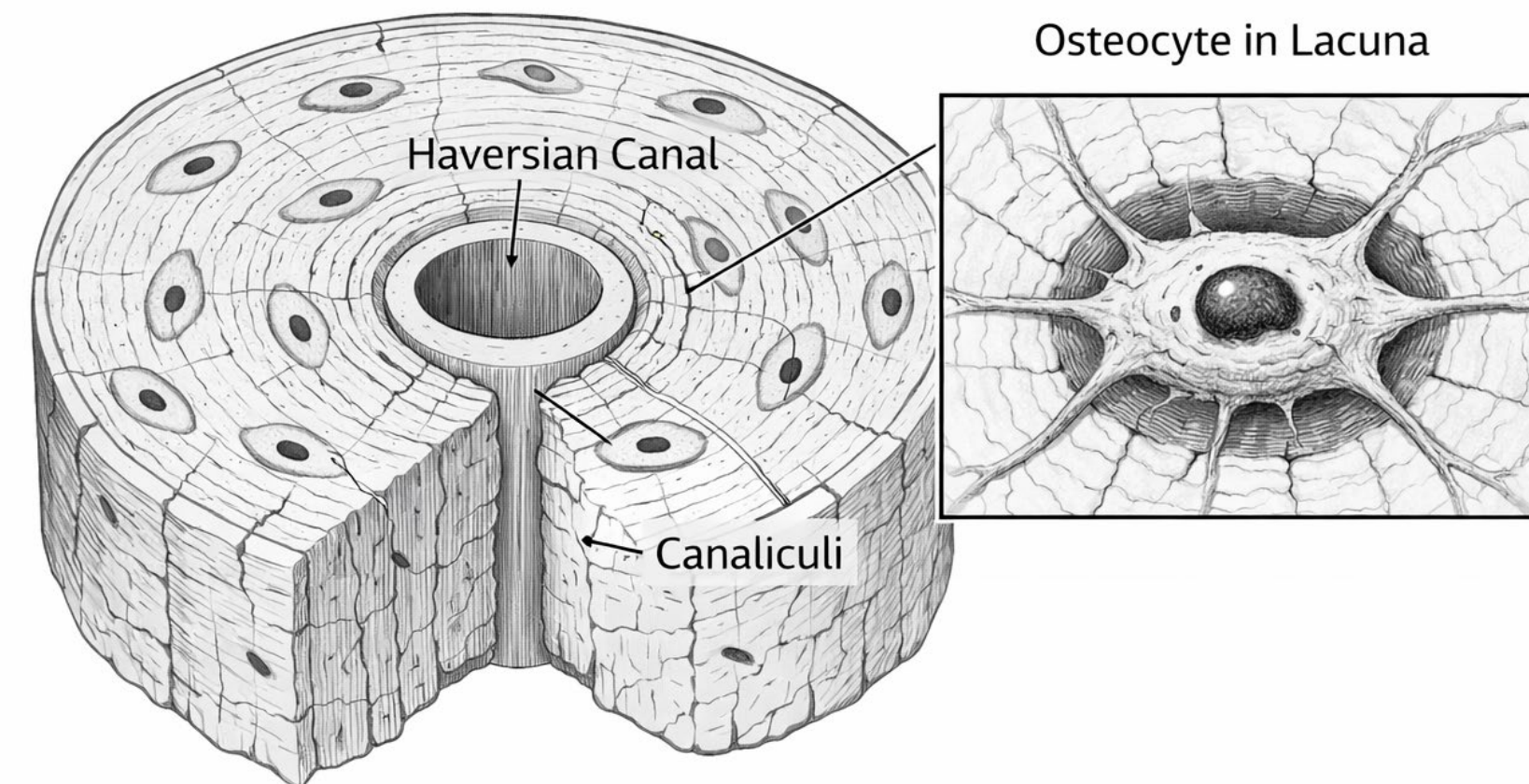
## Introduction

### Study Overview

- T1D increases fracture risk despite normal bone mineral density (BMD)
- Suggests deficits in bone quality at the microscale
- Hypothesis: peri-lacunar matrix is altered in T1D

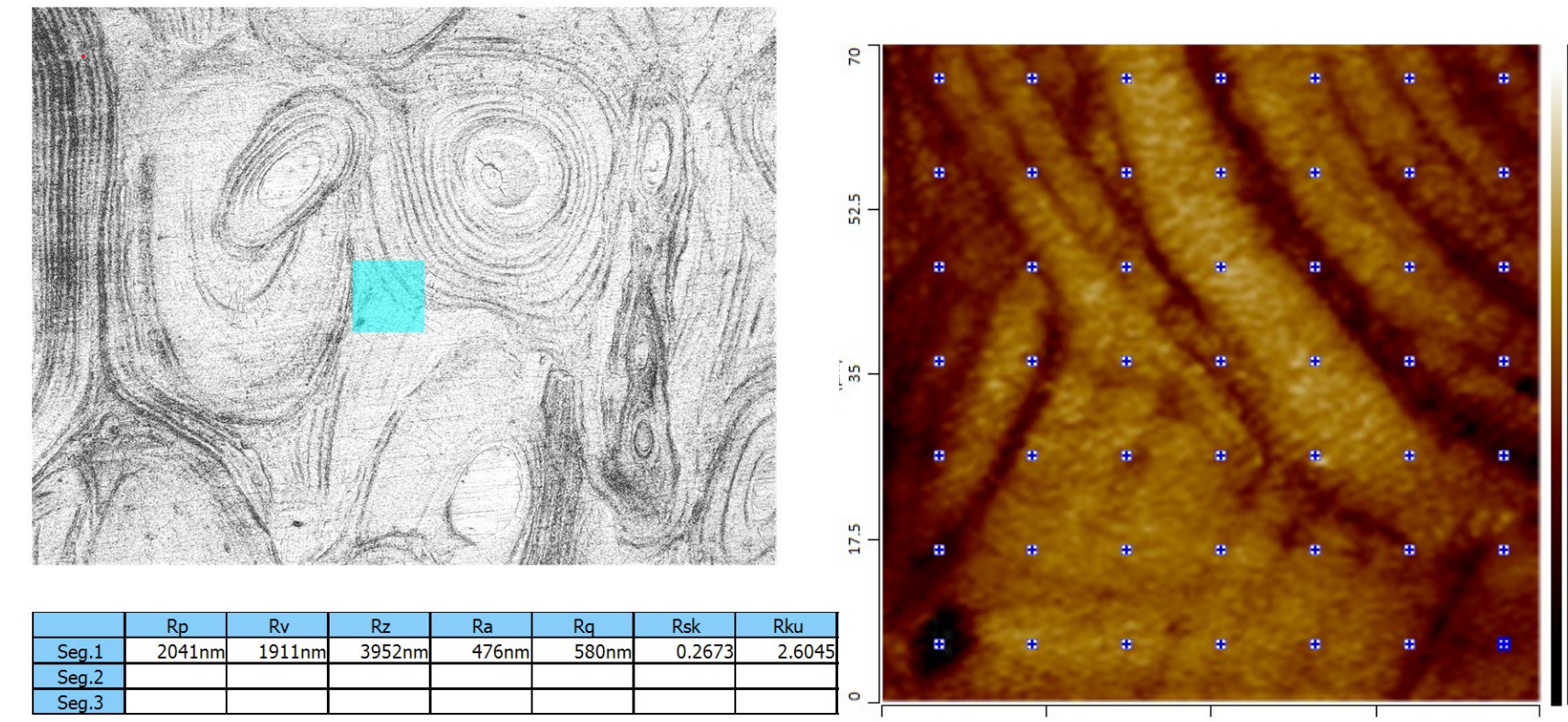
### Approach

- Measured nanoscale composition and mechanics around osteocyte lacunae
- Used 7×7 spatial arrays (10 μm spacing, ~70×70 μm region)
- Combined AFM-IR spectroscopy and nanoindentation



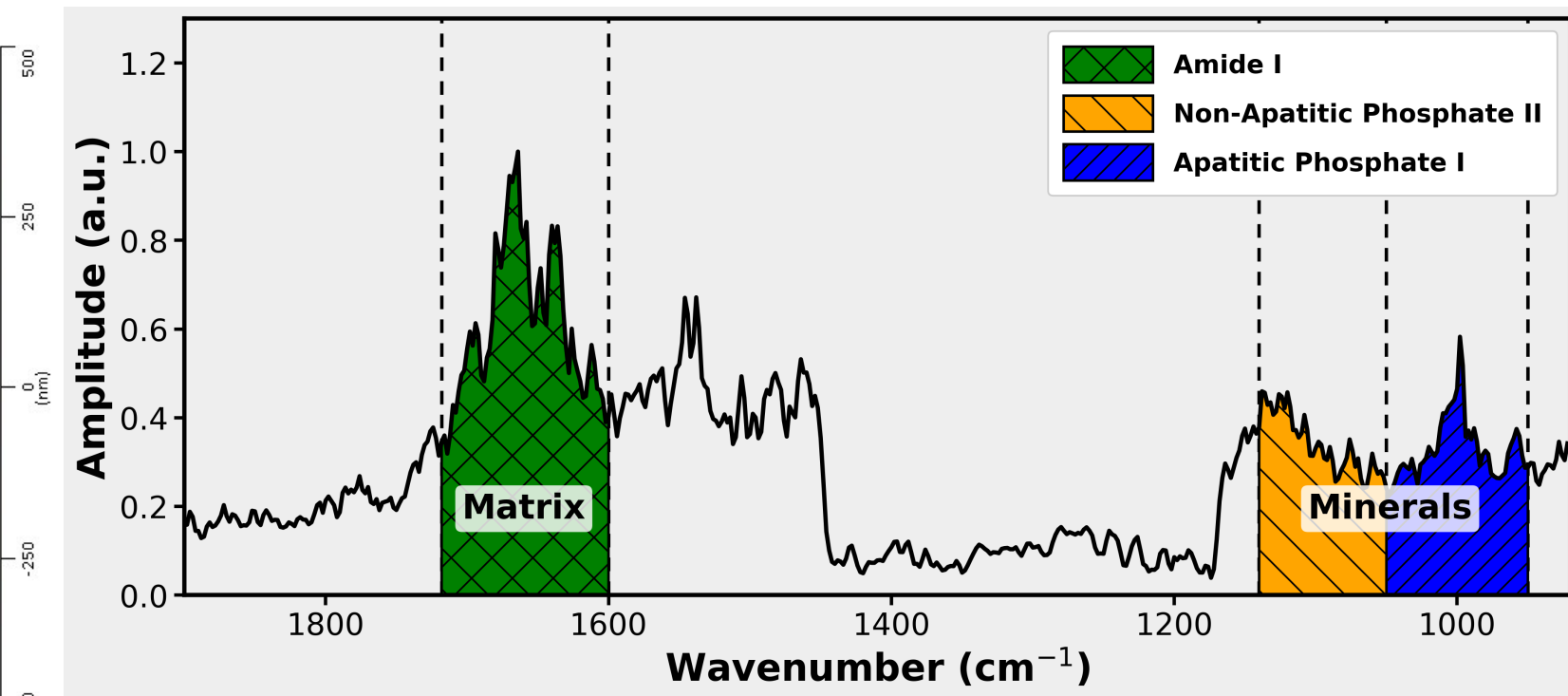
**Figure 1:** Cortical bone microarchitecture showing osteons, Haversian canals, and osteocyte lacunae connected by canaliculi. Osteocytes regulate the surrounding peri-lacunar matrix, which is the region analyzed in this study to quantify nanoscale compositional and mechanical changes associated with Type 1 Diabetes.

## Data Collection



**Figure 2:** (a) Keyence LSM optical image, “70×70 μm region; (b) Nano-IR height image showing Nano-IR collected spots; “70×70 μm region, 10 μm spacing”

## Localized IR



**Figure 3:** A typical localized Nano-IR spectrum collected from the lacunae array.

Phosphate bands represent mineral (blue - API, yellow - NAPII); Amide I represents collagen matrix (green - AI).

## Methods & Statistical Interpretation

### Measured Variables

#### IR-Based Variables

- API Intensity
- NAPII Intensity
- AI Intensity
- MMCA Ratio (API/NAPII)
- MMA Ratio ((API+NAPII)/AI)

#### Mechanical Variables

- Modulus
- Hardness

### Linear Mixed Model

#### Purpose:

- Tests spatial (distance) effects
- Tests group differences (case vs control)
- Tests interaction effects
- Accounts for clustering within samples

### Welch's t-test

#### Purpose:

- Compares case vs control groups
- Does not assume equal variance
- Evaluates group-level mean differences

### How were statistics used?

**Linear mixed models** were used as the primary analysis to evaluate spatial trends while accounting for repeated pixel-level measurements clustered within samples.

**Welch's t-tests** were used as a secondary analysis to compare case vs control groups without assuming equal variance.

**Together**, these approaches provide:

- spatial trend analysis
- group comparison
- complementary interpretation

## Results – Linear Mixed Model & Welch's T-test

### Spatial Variation in Bone Properties (LMM Results)

#### Linear Mixed Model Summary

Variable	Slope	p-value	Interpretation
API	+0.035	0.065	Weak trend
NAPII	+0.029	0.106	No effect
AI	+0.038	0.046	Small increase
MMCA	-0	0.797	No gradient
MMA	-0	0.744	No gradient
Modulus (GPa)	-0.012	<0.001	Strong decrease
Hardness (GPa)	-6.18e-4	0.003	Decrease

#### Interpretation

##### Key Findings:

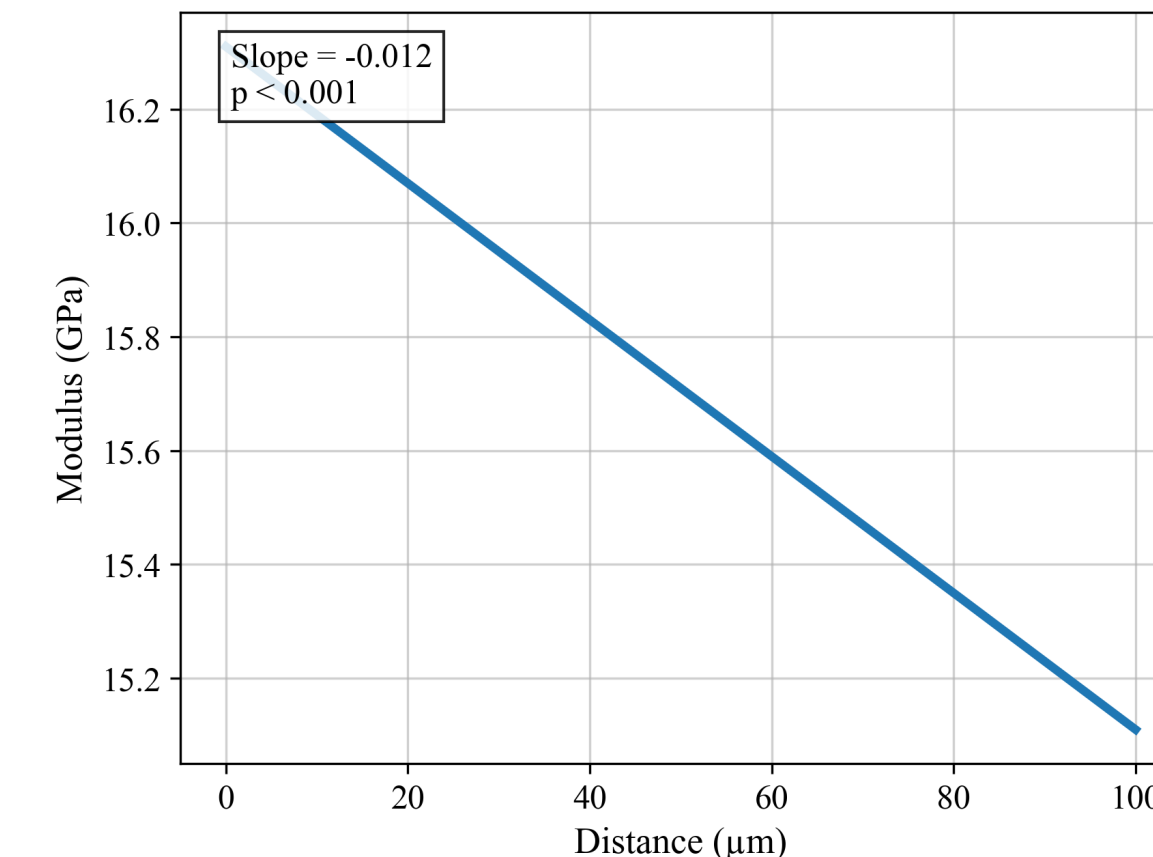
- Modulus and hardness significantly decrease with increasing distance from the lacuna.
- API and NAPII intensities show no significant spatial dependence, although PI trends upward.
- AI intensity shows a small but significant increase with distance.
- MMCA and MMA ratios show no significant spatial gradient.
- No significant group effects or group \* distances interaction effects were detected.

### Welch's t-test Summary

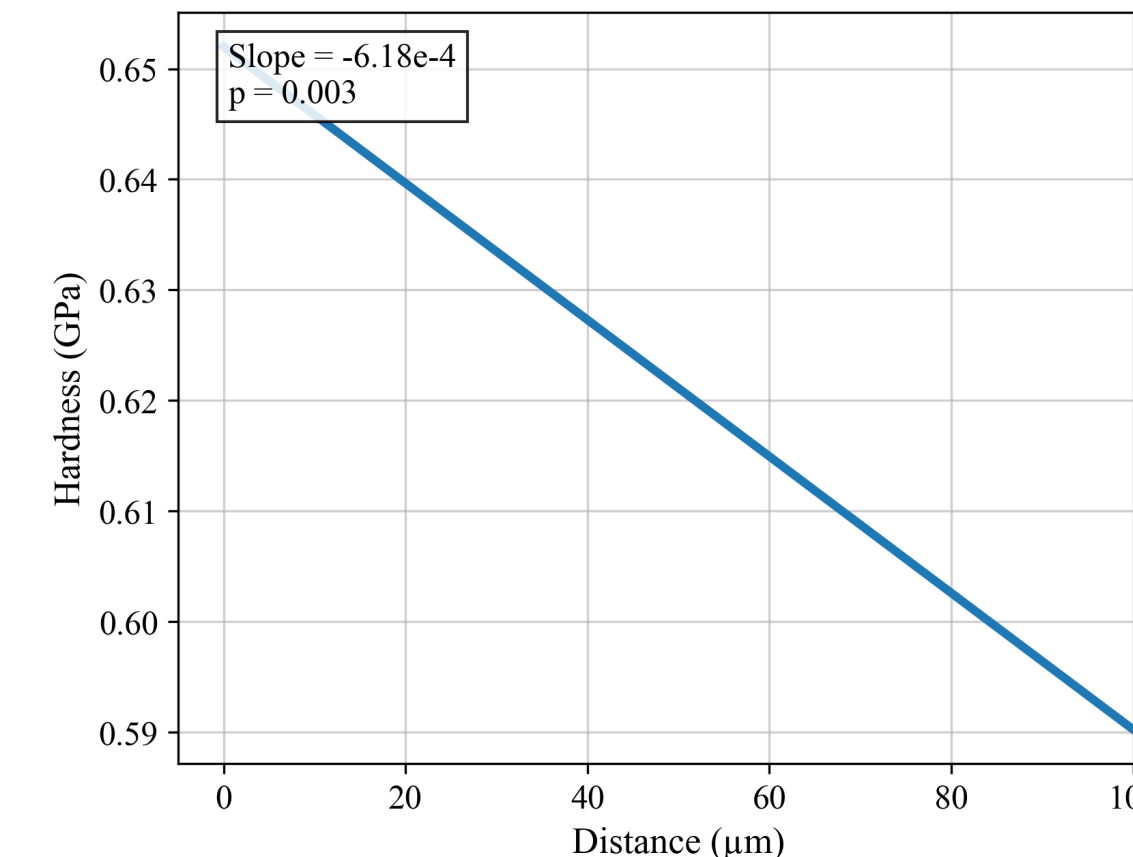
Variable	Case (T1D)	Control (non-T1D)	Relation	P value
API	26.5±14.77	24.62±14.05	↑	0.014
NAPII	24.25±13.13	24.87±12.89	↓	0.372
AI	33.33±16.37	29.83±15.74	↑	<0.001
MMCA	1.08±0.14	0.99±0.23	↑	<0.001
MMA	1.56±0.52	1.98±1.2	↓	<0.001
Modulus (GPa)	14.65±3.5	16.73±2.81	↓	<0.001
Hardness (GPa)	0.56±0.22	0.66±0.17	↓	<0.001

Mean ± Standard Deviation  
Note: Welch's t-test used for group comparisons (unequal variance)  
N = 30 lacunae (17 control, 13 T1D)

#### Modulus vs Distance



#### Hardness vs Distance



## Conclusion

1. Bone tissue in T1D exhibits reduced mechanical properties, with significant decreases in modulus and hardness.
2. These mechanical gradients occur with distance from the lacuna, but are not accompanied by strong spatial changes in bulk mineral composition. However, group-level differences indicate increased mineral crystallinity (MMCA) and altered mineral-to-matrix ratios (MMA) in T1D bone.
3. Together, these findings suggest that mechanical degradation in T1D is driven not by bulk compositional gradients, but by changes in mineral organization and matrix structure at the nanoscale.