Inverse correlation between trabecular bone volume and bone marrow adipose tissue in rats treated with osteoanabolic agents.
Conflicts of Interest and Funding

The authors have no conflicts of interests to declare

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- NIH/NIDDK R24 DK092759-01
- Harvard’s Skeletal Phenotyping Core NIH/NIAMS P30 AR066261
- Start-up funds from the Maine Medical Center Research Institute.
Outline

1. Background
2. Method Development: ImageJ Bone and BMAT Quantification
3. Effects of Sclerostin Antibody (Scl-Ab) and Human Parathyroid Hormone (hPTH) on bone and BMAT
Hypothesis: Scl-Ab and hPTH treatments would decrease overall adiposity and induce a negative correlation between BMAT and trabecular bone.
Increasing Trabecular Bone results in decreasing adiposity
Increasing Trabecular Bone results in decreasing adiposity
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Increasing Trabecular Bone results in decreasing adiposity.
Bone-Independent Adiposity Analysis

Tissue Area (T.Ar) = Trabecular Bone Area (Tb. B.Ar) + Marrow Area (Ma.Ar)
Marrow Area (Ma.Ar) = Tissue Area (T.Ar) - Trabecular Bone Area (Tb. B.Ar)
1. Developed ImageJ pipeline to test Scl-Ab and hPTH treatments effect on adiposity (adiposity/tissue area, or adiposity/marrow area) and bone.

2. Optimized parameters for adipocyte and trabecular bone quantification.
Adipocyte Quantification Method
Trabecular Bone Quantification Method

\[ T.Ar = Tb.B.Ar + Ma.Ar \]
\[ Ma.Ar = T.Ar - Tb.B.Ar \]
Scl-Ab and hPTH caused no inverse correlation between Ma.Ar adiposity and B.Ar after 26 weeks

Collectively, the osteoanabolic treatments showed a significant inverse correlations in the tissue area in the males after 26 weeks

No inverse correlations within the marrow area were established at this time point
Osteoanabolic agents decreased T.Ar Adiposity more significantly than Ma.Ar after 26 weeks.

hPTH significantly decreased Ma.Ar adiposity after 26 weeks.

*p<0.05 vs. Vehicle; ^p<0.05 vs. hPTH; †p<0.05, 4-week vs. 26-week; âp<0.05 Scl-Ab (3 mg/kg) vs. Scl-Ab (50 mg/kg). Data is shown as mean ± S.E.M. All analyses were performed as 2-way ANOVA + Tukey’s/Sidak’s multiple comparison tests.
hPTH decreased adipocyte number, but not size, in the Ma.Ar

*Marrow Area Male

- Vehicle
- Scl-Ab 3 mg/kg
- Scl-Ab 50 mg/kg
- hPTH 75 µg/kg/d

*\(p<0.05\) vs. Vehicle; h\(p<0.05\) vs. hPTH; T\(p<0.05\), 4-week vs. 26-week; A\(p<0.05\) Scl-Ab (3 mg/kg) vs. Scl-Ab (50 mg/kg). Data is shown as mean ± S.E.M. All analyses were performed as 2-way ANOVA + Tukey’s/Sidak’s multiple comparison tests.
Conclusion

1. Bone marrow adiposity quantification depends on if adipose tissue is normalized to T.Ar or Ma.Ar
2. Chronic Scl-Ab and hPTH caused a significant inverse correlation between tissue area adiposity and trabecular bone area
3. Ma.Ar adiposity was decreased by trabecular bone accrual only after chronic treatments with hPTH in males
4. The ImageJ platform designed and used here is useful for histology quantification
   - multiple stains- H&E, Von Kossa tetrachrome, Trichrome
   - bone marrow adipose
   - trabecular bone
   - cortical bone
   - white adipose tissue
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ImageJ Validation with OsteoMeasure

![ImageJ Validation with OsteoMeasure](image-url)
ImageJ Trabecular Bone Quantification Validation

Reprinted from Ominsky et al. 2015\[1\]

*p<0.05 vs. Vehicle; \(^{h}\)p<0.05 vs. hPTH; TP<0.05, 4-week vs. 26-week; \(^{A}\)p<0.05 Scl-Ab (3 mg/kg) vs. Scl-Ab (50 mg/kg). Data is shown as mean ± S.E.M. All analyses were performed as 2-way ANOVA + Tukey’s/Sidak’s multiple comparison tests.
Adiposity Correlations to Tb. B.Ar/T.Ar after 4 weeks of treatment

**Female**

Tissue Area

\[ Y = -0.008377X + 1.262 \]

\[ R^2 = 0.01116, p = 0.5166 \]

Marrow Area

\[ Y = 0.008167X + 1.223 \]

\[ R^2 = 0.00398, p = 0.6989 \]

**Male**

Tissue Area

\[ Y = -0.01588X + 1.553 \]

\[ R^2 = 0.1366, p = 0.0224^* \]

Marrow Area

\[ Y = 0.0004851X + 1.526 \]

\[ R^2 = 8.743e-005, p = 0.9556 \]
Adipocyte parameters/T.Ar

Female

Male

Vehicle  | Scl-Ab 3 mg/kg  | Scl-Ab 50 mg/kg  | hPTH 75 µg/kg/d

N.Ad/T.Ar. (#/mm²)

Adipose (%)

Adipose C (mm)

N.Ad/T.Ar. (#/mm²)

Adiposity (%)
Adipocyte parameters / Ma.Ar

**Female**

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**Male**

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* = p < 0.05 vs. control (Vehicle)
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