The importance of the GH/IGF-I axis for macroscopic and microscopic murine bone morphometry

1Schneider, P; 1Voide, R; 2Stampanoni, M; 4Donahue, L R; 5Müller, R
2Institute for Biomechanics, ETH Zurich, Zurich, Switzerland, 3Swiss Light Source, Paul Scherrer Institut, Villigen, Switzerland, 3Institute for Biomedical Engineering, ETH & University of Zurich, Zurich, Switzerland, 4The Jackson Laboratory, Bar Harbor, ME, USA
ram@ethz.ch

INTRODUCTION
Recent results showed that the intracortical canal network is a major contributor to local tissue porosity [1], and therefore, can directly be linked to measures of bone tissue quality. For this reason, we recently characterized the morphometry of mid-diaphyseal femoral cortical murine bone tissue on an organ and microstructural level [2] and provided strong evidence for a significant influence of the intracortical canal network on murine bone mechanics [3]. The goal of this study was to identify the relative importance of the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis for the macroscopic and microscopic bone morphometry, to test the hypothesis if there is an important interaction between sex steroids, the GH/IGF-I axis, and bone modeling.

METHODS
The little (lit) mutation mouse model was used to study the presence and absence of GH on the bone macro and microstructure. In total, 12 mice each (six female/male) of the four little mouse strains used (heterozygous: B6-lit/+ and C3.B6-lit/+; GH-deficient: B6-lit/lit and C3.B6-lit/lit) were raised at The Jackson Laboratory (Bar Harbor, ME, USA). On necropsy at the age of 4 months, femora were dissected and were stored in ethanol for further analysis. All animal procedures were approved by the Jackson Laboratory’s Animal Care and Use Committee.

We distinguished two different length scales. On the organ level, bone was characterized by its radial extension and volume distribution. On the tissue level, we described the canal network as microscopic phase of the intracortical porosity. The femoral mid-diaphyses of all mice were scanned using synchrotron radiation-based computed tomography (SRCT) at a nominal resolution of 3.5 µm [2]. For segmentation of the bone tissue on the macroscopic level, component labeling and morphologic operations were applied to the binarized data to get a solid representation of the mid-diaphysis. On the tissue level, the technique of negative operations were applied to the binarized data to get a solid representation of the mid-diaphysis. On the tissue level, the technique of negative imaging was applied to assess the intracortical canal network [2].

For morphometry on the macroscopic level, total volume (TV) enclosing both trabecular bone and the cortical bone tissue, bone volume (BV), bone volume fraction (BV/TV), cortical thickness (Ct.Th), and polar area moment (J) were assessed. On the tissue level, various morphometric indices for the canal network were calculated, where details regarding cannular measures are given in [2].

The 2 x 2 factorial design with two mouse pairs (B6-lit/+ & C3.B6-lit/+ and B6-lit/lit & C3.B6-lit/lit) implied the two-level independent factors mouse strain (B6-based and C3H-based) and sex (female and male). A two-way ANOVA was performed separately for the lit/+ and lit/lit pairs to test for significances in mouse strain and sex (p < 0.05). For cannular indices, where sex was an important factor (p < 0.1) according to the ANOVA, multiple unpaired t-tests including Bonferroni correction were conducted to locate significant differences (p < 0.05) between female and male mice within one strain. Moreover, correlation analysis was conducted between all indices to test for linear relationships.

RESULTS
The macroscopic indices (organ level) for all mouse strains showed that bone size (BV and Ct.Th) of GH-deficient lit/lit mice (B6: 0.60±0.04 mm³ and 133±5 µm, C3H: 0.75±0.07 mm³ and 216±15 µm) was smaller compared to heterozygous (lit/+ mice) (B6: 1.02±0.08 mm³ and 193±13 µm, C3H: 1.43±0.17 mm³ and 356±32 µm). Furthermore, B6-based mouse strains were generally smaller (low bone mass) in body size than corresponding C3H-based mouse strains (high bone mass). Moreover, ANOVA for the two mouse strain pairs lit/+ and lit/lit (data not shown) revealed distinct sex differences for heterozygous mice on the macroscopic level, whereas for GH-deficient lit/lit mice, this sex-specific macroscopic difference was removed (except for BV/TV).

On the tissue level, canal volume density (Ca.V/CTV) of the GH-deficient strains (B6-lit/lit: 1.8±0.6‰, C3.B6-lit/lit: 4.8±1.5‰) was reduced two to three times versus the heterozygous strains (B6-lit/+: 4.4±1.3‰, C3.B6-lit/+: 11.9±1.7‰).

Correlation analysis was performed for all morphometric indices. A mouse strain-specific asymmetry for the lit/+ strains was discovered for the element-based cannular indices (mean canal volume <Ca.V>, mean canal diameter <Ca.Dm>, mean canal length <Ca.Le>, and mean canal orientation <Ca.θ>) of C3.B6-lit/+ mice, which were mostly correlated with bone size (Table 1), while no linear relationship was detected between basic elements of the canal network and bone size for B6-lit/+ mice (Table 1). By contrast, this strain-specific asymmetry was removed for the homozgyous lit/lit strains and in addition, element-based cannular indices were all independent of bone size (data not shown).

<table>
<thead>
<tr>
<th>Strain</th>
<th>&lt;Ca.V&gt;</th>
<th>&lt;Ca.Dm&gt;</th>
<th>&lt;Ca.Le&gt;</th>
<th>&lt;Ca.θ&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6-lit/+</td>
<td>0.37</td>
<td>0.08</td>
<td>0.53</td>
<td>-0.39</td>
</tr>
<tr>
<td>C3.B6-lit/+</td>
<td>0.34</td>
<td>-0.02</td>
<td>0.56</td>
<td>-0.62</td>
</tr>
<tr>
<td>B6-lit/+</td>
<td>0.09</td>
<td>-0.07</td>
<td>0.13</td>
<td>0.09</td>
</tr>
<tr>
<td>C3.B6-lit/+</td>
<td>-0.01</td>
<td>-0.15</td>
<td>0.00</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

Table 1: Correlation between macroscopic and cannular morphometry. Statistically significant R-values (p < 0.05) are shown in bold.

DISCUSSION AND CONCLUSIONS
Independent of the GH/IGF-I axis, B6-based mouse strains were always smaller (low bone mass) than C3H-based mouse strains (high bone mass). In other words, the mouse strain-specific differences induced by the bone mass axis were independent of the GH/IGF-I axis. In contrast, sex-specific differences in macroscopic bone morphology was only present in lit/+ mice (with the exception of BV/TV). It is known that GH, which is secreted in a sex-specific pattern, is the single most important regulator of IGF-I production and beyond, that IGF-I and IGF-II are key mediators of sex steroid actions. Consequently, there may be an important interaction between sex steroids, the GH/IGF-I axis, and bone modeling [4]. In contrast, another group reported [5] that – based on skeletal parameters of IGF-I knockout (KO), IGF-II KO, and lit/lit mice assessed by dual X-ray absorptiometry (DXA) and peripheral quantitative CT (pQCT) – that the GH/IGF-I axis is not a major player in contributing to sex-specific effects on bone size. This implication cannot be supported by our results, where the absence of GH/IGF-I removed the detected asymmetry in the lit/+ system in terms of sex-specific macroscopic morphometric indices, which is directly indicative of the interaction between sex steroids, the GH/IGF-I axis, and bone modeling. An additional asymmetry induced by the GH/IGF-I axis was discovered when relating the macroscopic indices to the microscopic cannular indices. Whereas for both GH-deficient mouse strains (B6-lit/lit and C3.B6-lit/lit), overall cannular indices were significantly and positively related to bone size as introduced as a general scaling rule before [2], this symmetry no longer appeared in the lit/+ strains. In other words, the absence of GH removed the asymmetry in the strain-specific coupling of the canal network to bone size.

To our knowledge, this is the first study investigating the relative importance of the GH/IGF-I axis for macroscopic and microscopic cannular bone morphometry. In addition, it was supported that there is interaction between sex steroids, the GH/IGF-I axis, and bone modeling.

SIGNIFICANCE
Concurrent morphometric analysis of macroscopic and microscopic bone phenotypes as presented here will provide new insights in the assessment of bone quality on all levels of bone hierarchy.

REFERENCES