**Use of Ultra-high Resolution X-ray to Measure Tibia Length in Rodents**

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**Introduction**

Measurement of tibia length in mice and rats is commonly done as part of cardiovascular studies and is used to normalize heart weight in studies where cardiac hypertrophy may be observed. In comparison to body weight, which can continue to increase as mice age or decrease due to other conditions, tibia length remains stable once animals reach maturity and has been found to be a more accurate normalization standard (Use of tibia length to quantify cardiac hypertrophy: application in the aging rat (FC Yin et al., 1982).

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**Materials & Methods (continued)**

Once the bone is prepared measurements of tibia length are made and recorded on a paper record. For this study caliper measurements (considered the gold standard) were compared to x-ray measurements and also used in the calculation of heart weight to tibia length ratio that is used to monitor changes in heart mass in cardiovascular models. Statistical significance was calculated by using a paired T-test.

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**Results**

Tibia length was measured in mice and rats (n=10/group) undergoing cardiovascular model studies in vivo using Faxitron x-ray just prior to necropsy and by traditional caliper methods post necropsy. All animal model studies were conducted under IACUC approval. For x-ray imaging mice or rats were anesthetized with isoflurane/oxygen and imaged under anesthesia in the Faxitron Ultrafocus unit (Faxitron Biopics LLC, Tuscon, AZ). Measurements were calibrated using ceramic calibration blocks (Mitutoyo, Aurora, IL). On-screen measurements were then used to create a correction factor to account for differences due to magnification from plate to surface distance. The correction factor was on average 0.968.

Post necropsy, technicians disarticulate the lower leg and completely strip the soft tissues from the tibia, similar to creating a bone for histological preparation.

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**Discussion**

Collection of tibias at animal necropsy and bone preparation to obtain accurate measurements with calipers is both time consuming and difficult. To get an accurate caliper measurement of tibia length post necropsy, technicians are essentially creating a bone ready for histological preparation when we are not interested in a histological endpoint. We proposed to replace caliper measurements with ultra-high resolution x-ray imaging, which would allow for in vivo measurement or removal of the leg, followed by x-ray. This allows us to collect tibia length without having to undergo and further tissue trimming. This saves time, is more efficient, and we get an image with a scaled measurement. The caliper method creates a paper record that is prone to more typographical errors. X-ray measures are consistently higher than caliper data, but is also more consistent across groups.

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**Conclusion**

Use of x-ray to measure tibia length in rodents represents a replacement of the caliper method and ultimately is more efficient. Additionally, x-ray has the added benefit of allowing for in vivo multiple measurements to be collected throughout the duration of a study, particularly if rodents are in a growth phase. X-ray measures for tibia length can be done in vivo or ex vivo depending on study needs.

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**References**