Using Time-Lapse HR-pQCT for Bone Turnover Classification in CKD-MBD Patients

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Abstract

Patients with chronic kidney disease – mineral and bone disorder, a disease that results in a high risk for bone fractures, can be classified based on the rate of total bone turnover. Currently, bone turnover rates are determined from iliac crest bone biopsies followed by quantitative histomorphometry. However, this method is painful, invasive, and performed in an area not typically prone to fractures. In this ongoing prospective study, we explore the use of time-lapse high resolution peripheral quantitative computed tomography (HR-pQCT) imaging as a non-invasive method to determine bone turnover in patients. Fifteen participants are expected to participate in this study. To date, six participants were recruited to undergo HR-pQCT scans at four different timepoints with two-month intervals. Three scans will be performed at the first timepoint and one scan will be performed at the three following timepoints. The three repeat baseline scans and each of the follow-up scans underwent time-lapse analysis to determine total bone turnover. For this thesis project, the objective was to establish methodology for this study, including determine the time-lapse threshold needed to detect <0.5% reproducibility differences, determine the more robust image processing pipeline between two processing techniques, determine the least significant change (LSC) from baseline repeat scans, and determine if there are detectable time-lapse changes in total bone turnover at two-months. The preliminary results determined that using a Laplace-Hamming image processing pipeline performed better than the standard analysis protocol, determined that a threshold of 525 mgHA/ccm led to <0.5% reproducibility differences, demonstrated a LSC of 0.088 and 0.040 for the radius and tibia respectively, and showed that after two months, time-lapse HR-pQCT could measure *in vivo* total

bone turnover in patients but not greater than the LSC values. Since determining the optimal time to scan was an objective of this study, the continuation of the study and scanning at longer timeframes will uncover the period with which we can measure bone turnover greater than the LSC. The continuation of this study will also elucidate the relationship between time-lapse HR-pQCT results and the gold standard of bone biopsies and verify our initial conclusions.

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Introduction

Chronic kidney disease – mineral and bone disorder (CKD-MBD) is defined as "a systemic disorder of mineral and bone metabolism due to CKD" [1]. This disease manifests by either one or a combination of abnormalities of blood bone biomarkers, bone turnover, or vascular or other soft tissue calcification [1]. The most common classification method for CKD-MBD type is by high and low bone turnover, both of which can lead to low bone mass and a high risk for fractures. Determining turnover type is critical for treatment as the current treatment options are diametrically opposite depending on whether a patient would benefit from an increase or decrease in bone turnover rate.

Ideally, noninvasive techniques would be preferred to determine bone turnover type. Dual energy x-ray absorptiometry (DXA), the conventional imaging tool used to measure bone mineral density (BMD), typically shows low BMD measures in patients with CKD-MBD due to abnormal turnover rates. However, DXA is unable to differentiate the specific bone turnover type. Another common option to determine bone turnover is looking at blood biomarkers such as fibroblast growth factor 23 (FGF-23) and parathyroid hormone (PTH). However, FGF-23 and PTH are elevated in cases of both high and low bone turnover due to renal failure, limiting the capacity to differentiate turnover type [2,3].

The gold standard in determining bone turnover rates in CKD-MBD patients is the iliac crest bone biopsy with tetracycline double-labeling and quantitative histomorphometry. To date, this method is the only way to measure osteoid thickness, bone formation, and resorption rates which are necessary for diagnosing osteomalacia or adynamic bone disease. However, this procedure is limited in use due to its invasiveness and availability [2,3]. Because of this, it is commonly performed only once and limited to one location. This requires researchers to assume

that bone remodeling at the iliac crest is representative of other skeletal sites, which has yet to be verified [2,4]. The iliac crest is also not a weight-bearing site and not typically a region at high risk for fractures due to osteomalacia or adynamic bone disease. Additionally, the processing time to perform quantitative histomorphometry can take up to three months so this method is not ideal for rapid decision making for patient treatment [2]. Lastly, identification of many of the histological features, such as resorption cavities and osteoid seams, remains a subjective process.

High-resolution peripheral quantitative computed tomography (HR-pQCT) is a noninvasive, low radiation dose research imaging modality that measures BMD, bone geometry, and bone microarchitecture of patients *in vivo* at peripheral anatomic sites, most commonly the distal radius and tibia. These anatomical sites are common areas of fracture in those with osteomalacia and adynamic bone disease and the tibia is weight bearing, which can provide additional information on how loading impacts the bone. The second-generation HR-pQCT scanner, the XtremeCT II, images at a nominal isotropic voxel size of 61um. By acquiring images at this spatial resolution, we can better visualize and differentiate between cortical and trabecular bone, which are approximately 100um in the smallest dimension, and extract microarchitecture parameters [7]. Recently, researchers have developed time-lapse HR-pQCT that utilizes sequential HR-pQCT scans at the same anatomical site over time [5]. Sequential scans are spatially aligned using 3D registration techniques, and areas of bone formation and resorption are identified and quantified as a percent of baseline bone volume [5]. By calculating the bone formation and resorption fractions at different time points, we can utilize time-lapse analysis to calculate total bone turnover rates to classify patients as high or low bone turnover. This method has the potential to address some of the major limitations of bone biopsies. Not only is this method noninvasive, but it also allows for longitudinal measurements to track patient disease progression. However,

prior to this study, time-lapse had not been applied to CKD-MBD, nor had it been validated against the gold standard bone histomorphometry.

The goal of this ongoing study is to determine whether time-lapse HR-pQCT can be used as a noninvasive tool to identify bone turnover type in patients with CKD-MBD. Specifically, the aims of this prospective experimental study were to determine (1) the least detectable *in vivo* bone turnover using the XtremeCT II scanner, (2) the shortest period with bone turnover greater than the least detectable value in CKD patients, and (3) whether total bone turnover by time-lapse HR-pQCT is associated with bone turnover by histomorphometry of iliac crest biopsies. For this thesis project, there was additional focus on developing the methodologies to address these aims including determining a time-lapse threshold parameter and image processing and analysis pipeline.

Methods

Participants

Fifteen individuals with chronic kidney disease and on dialysis will be recruited. Those undergoing antiresorptive or anabolic treatment and those that have had a kidney transplant will be excluded. Ten individuals will undergo the workflow for Aims 1 and 2 as shown in **Figure 1** below. The remaining five will undergo the workflow for Aim 3. Those in this group also need to be referred for biopsy prior to study inclusion.

Study Design

At the initial visit, all participants will consent to and undergo three separate HR-pQCT scans at each anatomical site (radius and tibia) to determine the least detectable *in vivo* bone turnover. Between each repeat scan, participants will be removed from the scanner and repositioned. Participants contributing to Aim 3 will also get additional blood work during the

baseline visit. Aim 1 and 2 participants will have three follow-up visits at 2-month intervals during which additional HR-pQCT scans will be acquired. Aim 3 participants will only have two follow-up visits followed by a biopsy. The follow-up HR-pQCT scans for Aim 2 will be used to determine the shortest follow-up period necessary to quantify bone turnover. The follow-up scans for Aim 3 will be used to compare time lapse HR-pQCT turnover measures with gold standard biopsy and histology measures. A summary of the study design is outlined in **Figure 1**.

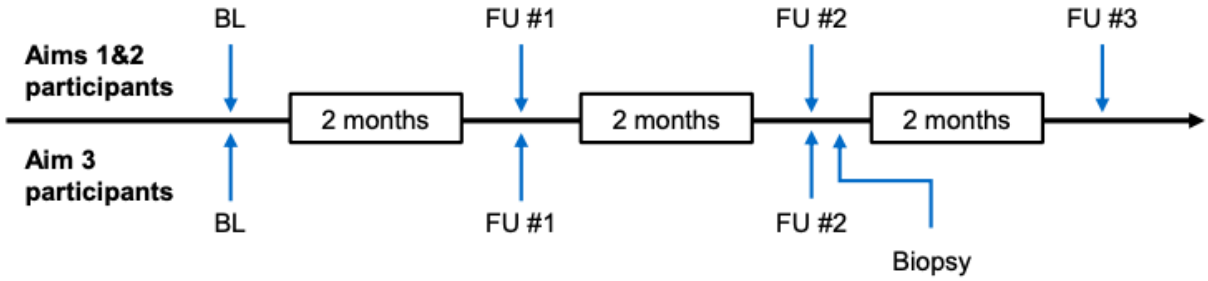

***Figure 1**. Study design workflow*

Image Acquisition

HR-pQCT images will be acquired using the XtremeCT II scanner and Scanco Medical's standard *in vivo* protocol at the radius and tibia. This protocol produces images with 61um isotropic voxels. At each timepoint and anatomical site, an ultra-distal scan will be acquired. The location of this scan will be set to 4.0% and 7.3% of the overall bone length for the radius and tibia respectively for the Scanco Medical's standard *in vivo* protocol. The scan time will be approximately two minutes and the radiation dose per scan was 5uSv.

Image Processing

All images will be segmented to extract the cortical and trabecular compartments. This is done through semi-automated contouring of the periosteum and the endosteum. **Figure 2** shows an example of the periosteal and endosteal contours at the tibia and radius respectively. Once the

cortical and trabecular bone are segmented, microarchitecture parameters will be extracted using Scanco Medical's standard protocol and internal UCSF Laplace-Hamming protocols.

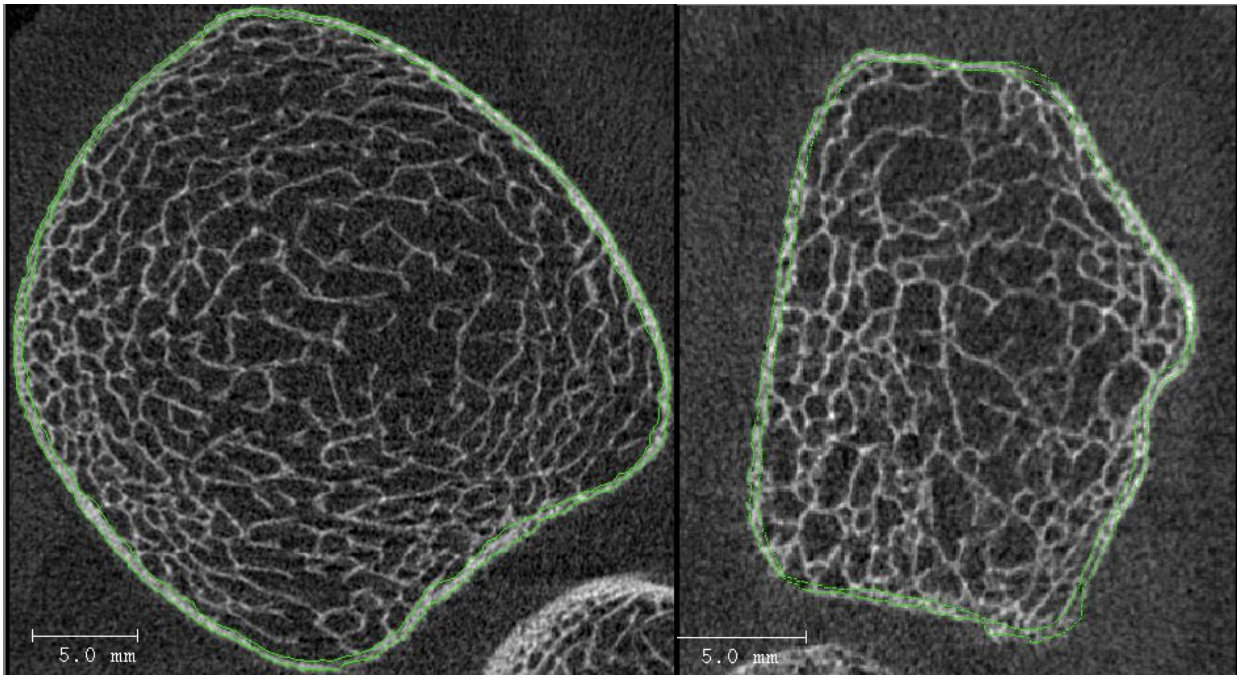

***Figure 2**. Example of periosteal and endosteal contours.*

Parameter Study

To determine the optimal global threshold for time-lapse analysis, twenty-six scans of ultra-distal radius and tibia phantoms underwent time-lapse analysis at global thresholds of 375 mgHA/ccm to 550 mgHA/ccm with increments of 25 mgHA/ccm. The phantoms consisted of healthy cadaver tissue with intact bone marrow embedded in a polymer resin with x-ray radio-opacity similar to that of soft tissue [8]. The threshold that resulted in a total bone turnover of <0.5% was used to analyze the *in vivo* scans from the CKD-MBD cohort.

Time Lapse Analysis

Baseline and follow-up scans will be rigidly registered using the XtremeCT II scanner software (IPL, Scanco Medical AG). Only the volumes common to all timepoints will be analyzed to determine bone turnover. The registration process will create a transformation matrix to bring

all follow-up scans into the coordinate system of the baseline scan. Once registration is completed, each pair of baseline and follow-up scan will undergo voxel-by-voxel subtraction creating a grayscale image representing the voxel-wise differences in bone density between baseline and the different follow-up timepoints. After, the image will be filtered using two methods to reduce artifacts. First, a global threshold of 525 mgHA/ccm will be applied to remove differences resulting from noise or registration errors. A second filter will be applied to remove small clusters of voxels (<5 contiguous voxels) that characterize formation or resorption zones to exclude additional noise artifacts. From the denoised image, the bone formation and resorption fractions will be calculated by dividing the number of voxels for formation or resorption by the baseline total bone voxels. Total bone turnover (TBT) is the summation of formation and resorption voxels divided by the baseline total bone voxels. **Figure 3** shows examples of bone formation fraction, bone resorption fraction, and total bone turnover taken from a previous study comparing a baseline image to a two-year follow-up image.

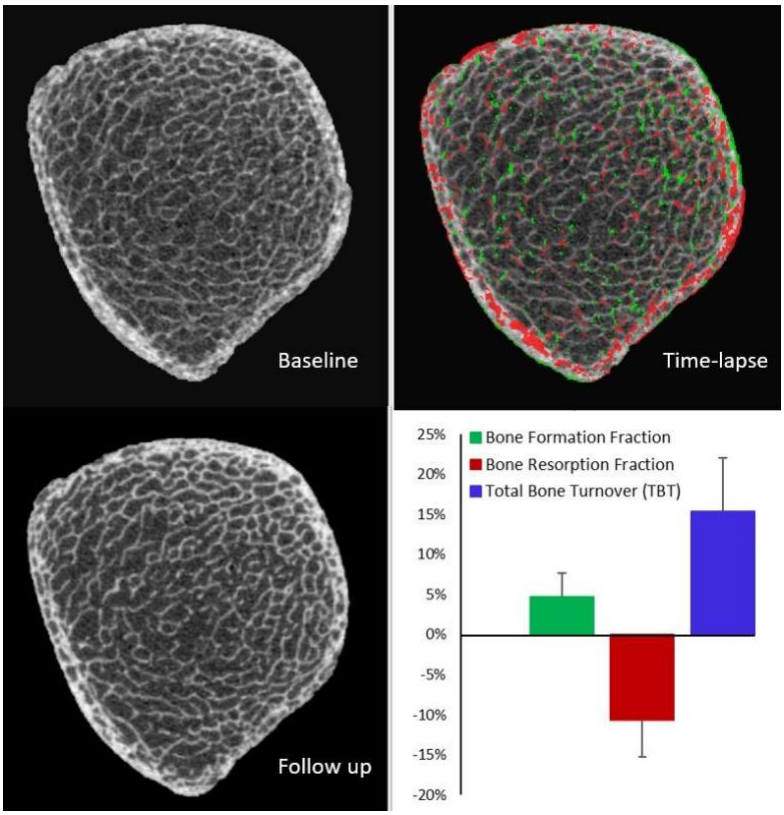

***Figure 3**. Example of time-lapse analysis.*

Statistical Analysis

For the baseline scans (Aim 1), time-lapse metrics will be calculated for each repeat scan with respect to the original scan at each location at each anatomical site. The mean and standard deviation will be calculated at each location to determine the coefficient of variation (CV) and the precision error will be calculated as the root mean square coefficient of variation (RMSCV); the equation is shown in **Equation 1**.

$$RMSCV = \sqrt{\frac{\sum_{i=1}^{N} CV^2}{N}}$$

***Equation 1**. Equation to calculate the precision error. N is the total number of samples.*

The RMSCV will then be used to calculate the least significant detectable change (LSC = 2.77*RMSCV) which is reported for each anatomical site. The LSC represents the smallest

difference in sequential measurements that can be considered a real change and not due to chance [6]. For follow-up scans (Aim 2), time-lapse metrics will be calculated with respect to the baseline scan at each anatomical site. We will then compare the total bone turnover from the follow-up scan to the LSC. For Aim 3, time-lapse metrics will also be calculated for each of the two-month follow-up scans with respect to the baseline scan. We will also compare the total bone turnover from the follow-up scans to the LSC. Lastly, we will perform a linear regression to calculate the association of the time-lapse HR-pQCT to the gold standard of bone biopsies with quantitative histomorphometry.

Current Work

To date, six individuals have been recruited for Aims 1 and 2. All recruited participants have completed their baseline scans, four participants have completed their first follow-up scans, and one participant has completed their second follow-up scan. Of the participant scans that have been acquired, four baseline scan sets and three two-month follow-up scans have undergone image processing, time-lapse analysis, and statistical analysis. The parameter study was also completed, and the resulting threshold was used in the time-lapse analysis workflow.

Results

Image Processing

All images were processed using both Scanco Medical's standard analysis protocol and UCSF's Laplace-Hamming image processing protocol. **Figure 4** shows the output of each processing protocol.

At both anatomical sites, we observed areas of less trabecular bone detection, as indicated by the red arrows in **Figure 4**, when using the standard protocol. We also see larger cortical pores and, specifically in the images of the radius, breaks in the cortex using this processing workflow.

The Laplace-Hamming protocol results in a thicker and uninterrupted cortex with smaller cortical pores. We also see thicker and continuous tracks of trabecular bone in the areas where no bone was detected in the standard processing workflow.

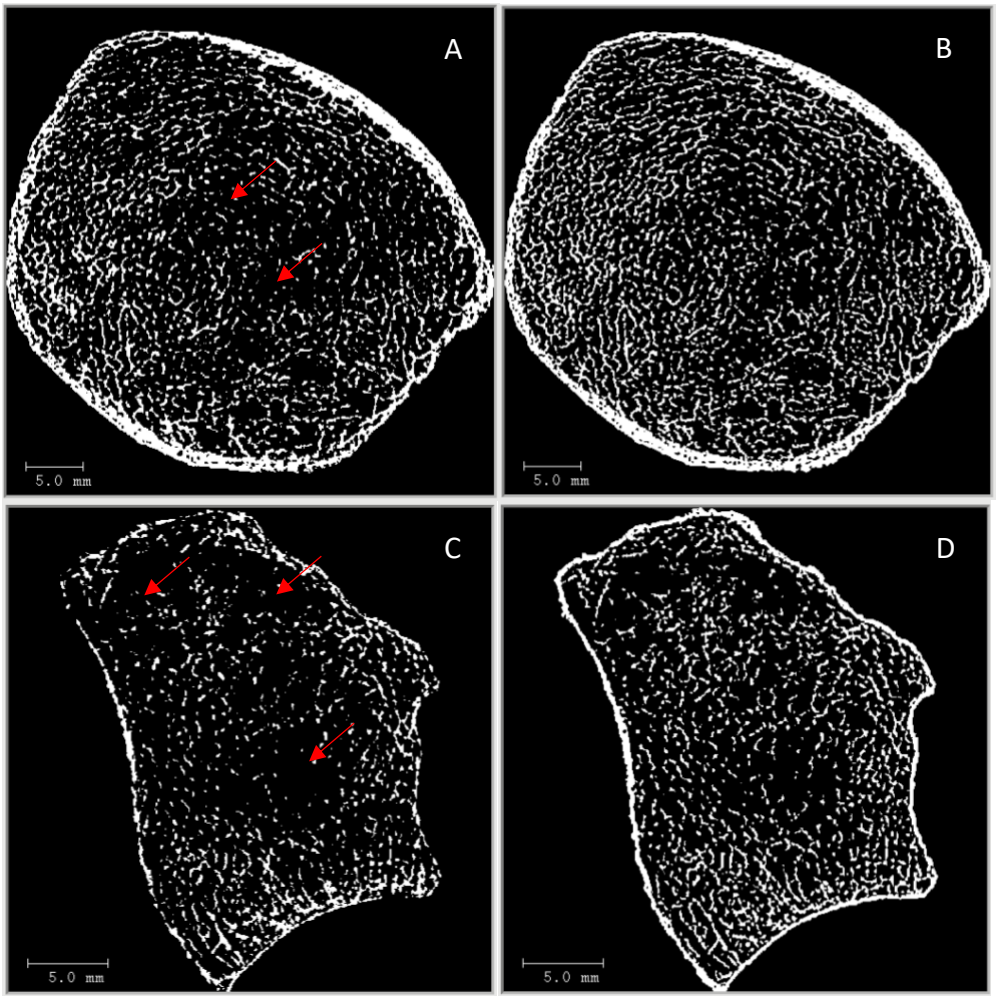

Figure 4. *Comparison between standard and Laplace-Hamming protocols for CKD-003. Images A and C are the results of the standard protocol. Images B and D are the results of the Laplace-Hamming protocol. Images A and B are of the tibia and image C and are of the radius*

Parameter Study

Figure 5 shows a plot of the average total bone turnover at each of the global threshold values. The threshold that is closest to the target total bone turnover value of <0.5%, as indicated by the green circle in **Figure 5**, is 525 mgHA/ccm.

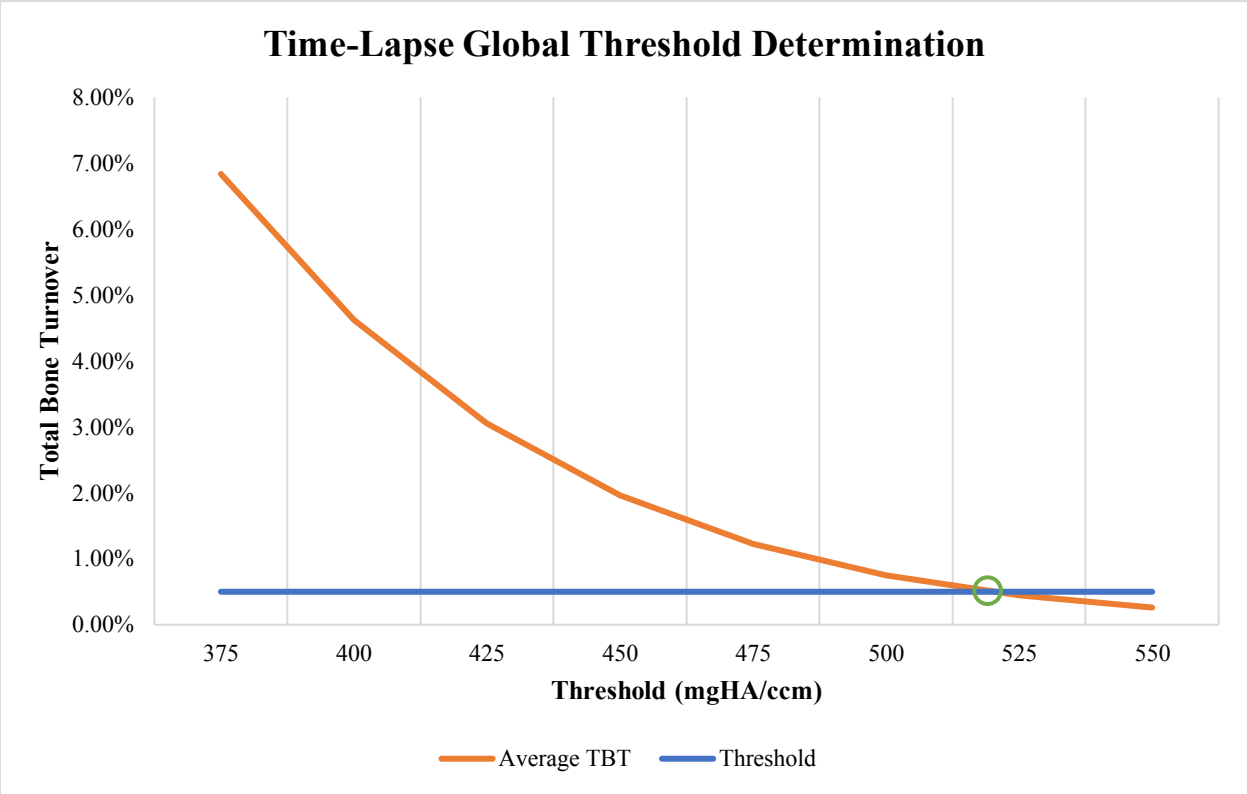

***Figure 5**. Parameter study results.*

Least Significant Change

After performing time-lapse analysis on the three repeat scans from four of the participants' initial visits, we calculated the mean TBT, bone formation fraction (BFF), and bone resorption fraction (BRF) at each anatomical site. From the means, we calculated RMSCV which was then used to calculate the LSC for TBT, BFF, and BRF. **Table 1** shows the mean, RMSCV, and LSC values for each parameter at each anatomical site.

***Table 1**. Least Significant Change Results*

Location	**TBT**			**BFF**			**BRF**		
	Mean	**RMSCV**	**LSC**	**Mean**	**RMSCV**	**LSC**	**Mean**	**RMSCV**	**LSC**
Radius	0.073	44%	0.088	0.034	38%	0.036	0.039	48%	0.052
Tibia	0.028	51%	0.040	0.014	48%	0.019	0.014	54%	0.021

Two-Month Follow-Up

To evaluate the time-lapse results after a two-month period, we compared the two-month total bone turnover values to the LSC values for three participants at each anatomical site. **Table 2** shows the individual results for each participant and each anatomical site. For both the radius and the tibia, the measured two-month total bone turnover was less than the LSC value except for Participant 3 at the tibia.

***Table 2**. Two-Month Time-Lapse Results*

Participant	Location	TBT
CKD_001	**Radius**	0.0393
	Tibia	0.0132
CKD_002	**Radius**	0.0647
	Tibia	0.0106
CKD_003	**Radius**	0.0149
	Tibia	0.0453

We also compared the reproducibility TBT values, which represents the error due to scan variability, to the two-month TBT values for the radius and tibia. **Figure 6** and **Figure 7** show the results for each participant at the radius and tibia respectively. The horizontal blue lines in each figure denote the LSC for TBT. At the radius, for Participants 1 and 2, their TBT increases over the two-month period, but not above the LSC threshold. For Participant 3, their reproducibility TBT was above the LSC line and decreased after two-months. At the tibia, we observed a reproducibility TBT at the LSC line with a decrease in TBT after two months for Participant 1. For Participants 2 and 3, we observed no changes. Also, for Participant 3, the longitudinal TBT was above the LSC threshold, but the reproducibility TBT was also above the LSC threshold.

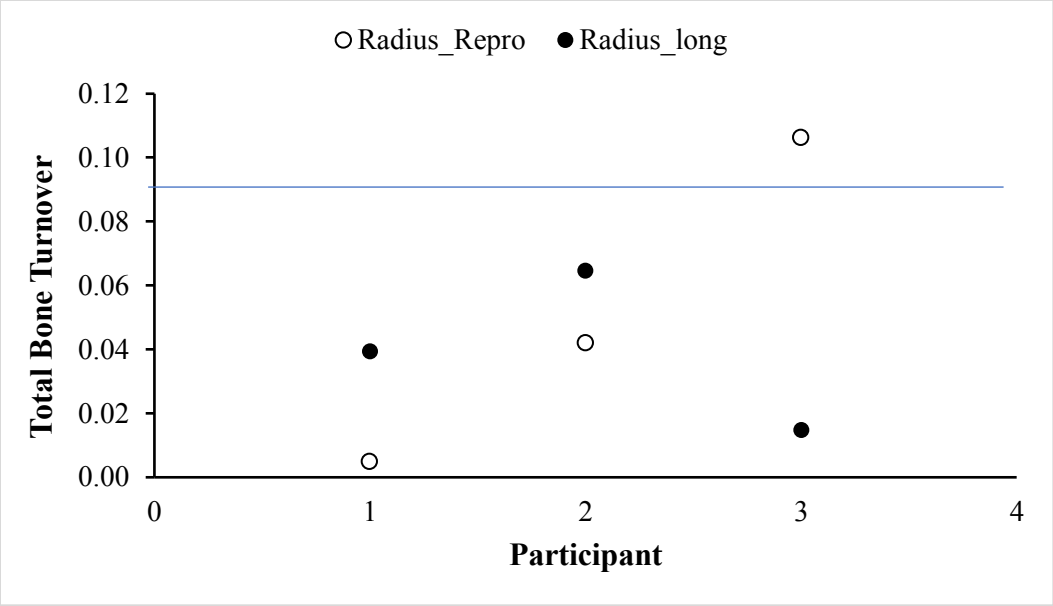

Figure 6. *Comparison of reproducibility and longitudinal total bone turnover at the radius.*

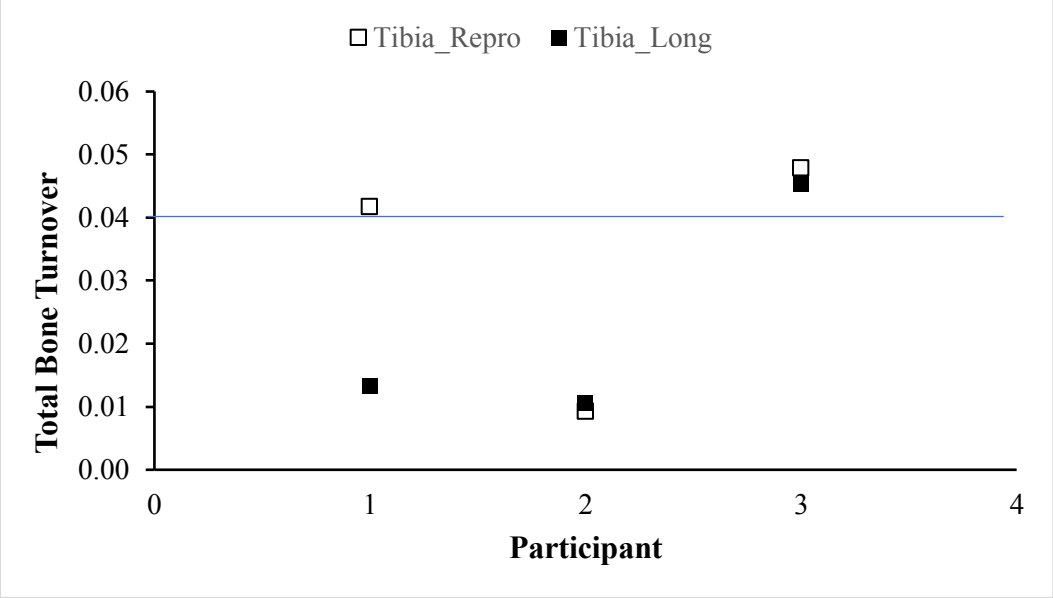

Figure 7*. Comparison of reproducibility and longitudinal total bone turnover at the tibia.*

Discussion

Prior to this study, HR-pQCT time-lapse analysis had not been applied to CKD-MBD. At this point in the study, we aimed to improve the image processing pipeline by comparing different image processing and analysis protocols and performing a parameter study, determine the LSC for

total bone turnover, and evaluate if two-months can detect total bone turnover greater than the LSC.

When comparing both the image processing techniques that we implemented, the Laplace-Hamming filtering protocol fit the needs of this study better as it resulted less data loss than the standard analysis process. To understand what was causing the differences in outputs, we performed a root cause analysis and determined that the Gaussian filtering step was smoothing the images such that bone edges were missed or misidentified. The Laplace-Hamming filtering identified the bone surface through edge detection which resulted in better identification of the fine detail with the trabecular compartment and the pores and edges in the cortical compartment. This technique worked well for the ultra-distal scan sites, but not as well for the distal scan sites, which prompted us to exclude the distal images at this time. Future work will need to be done to refine the Laplace-Hamming image processing workflow for distal radius and tibia images.

In analyzing the data for Aim 1, we hypothesized an average total bone turnover value and LSC of zero for the scans taken at baseline. However, we see that there is some variability between the scans taken on the same day as denoted by our measured LSC value. This can be attributed to differences in patient positioning and reference line placement, as we removed the participant from the scanner between repeat scans, and segmentation corrections. All of these could have added to the slight variations between the scans. Additionally, patient motion during the scan could have impacted registration and led to an increase in measured differences between baseline and repeat scans. Motion could also impact the measured bone mineral density within the bone leading to artificial differences between the scans. Lastly, inherent variations in scan quality could impact bone detection between the baseline scans leading to variability in identifying changes between

repeat scans. Further refinements to our image processing and analysis pipeline will likely be performed to address the high variability between the scans.

For all participants and anatomical sites at the two-month timepoint, all but one measurement identified using the XtremeCT II scanner were not greater than the LSC value. For the one participant and site that had a two-month TBT above the LSC threshold, the corresponding reproducibility TBT was also above the LSC threshold. Because if this observation, we cannot conclude that this measurement is a significant change in TBT. This result is a product of the high variability seen within our baseline repeat scans.

For two participants at the radius, we observed increases in TBT after two-months, but they did not cross the LSC threshold. This could be due to two scenarios. First, turnover in CKD-MBD over a two-month period may be too small to detect in individual participants. By testing different lengths of time between scans, we are hoping to capture the point when measured total bone turnover is greater than LSC. Analysis of the scans from the second and third follow-up visits will allow us to see if this hypothesis is true. Second, participants that have had their follow-up scans analyzed are those with low bone turnover. This would skew our results and lead us to believe that a two-month period is too short when it is possible that it is adequate for high bone turnover patients. Further clinical evaluation would need to be performed to confirm total bone turnover type for these three participants. Additionally, increasing our sample numbers could help normalize our sample population to have a more even split between high and low bone turnover participants. Lastly, further research in the percentages of patient with CKD-MBD that have high bone turnover versus low bone turnover would help elucidate what we should expect from our study population.

For all other participants and anatomical sites, we observed either decreases or no changes in TBT. These observations are also impacted by our high variability between baseline repeat scans. To ensure accurate longitudinal measurements, as mentioned above, further refinements to our image processing and analysis pipeline will need to be performed to address the high variability between baseline scans.

We also note that our investigation is focused on the ability to identify change in an individual, a clinically important metric. To assess the usefulness of these measurements for research purposes would require consideration of the variability due to true between-subject variability and that due to within-subject random measurement error, e.g., calculation of the intraclass correlation coefficient.

One additional challenge that we faced when conducting this study was recruitment. Because our cohort of patients already has a significant time burden of going into the clinic for dialysis multiple times a week, it was difficult to persuade prospective study participants to schedule an additional visit to the clinic for imaging. This resulted in a small sample size and led to the omission of comparing time-lapse results to the gold standard of bone biopsies with quantitative histomorphometry at this point in the study. This objective will be explored beyond the scope of this thesis.

Conclusion

At this early stage in the study, we were able to measure total bone turnover values after a two-month interval, but these were not above the LSC value at each anatomical site. We selected our image processing technique to best fit the needs of this study. However, although we were able to observe changes total bone turnover, it is still unclear what the optimal length of time is between scans in order to identify change in an individual. We also have not yet acquired biopsies so cannot

compare our results to the gold standard of bone biopsies with quantitative histomorphometry. Lastly, we do not know what the time-lapse thresholds are to classify a patient as either high or low bone turnover. The continuation of this study will elucidate any relationships between bone biopsies and time-lapse HR-pQCT and verify some of the initial conclusions we extracted from the current dataset.

References

[1] Moe S, Drüeke T, Cunningham J, et al. Definition, evaluation, and classification of Renal Osteodystrophy: A position statement from kidney disease: Improving global outcomes (KDIGO). *Kidney International*. 2006;69(11):1945-1953. doi:10.1038/sj.ki.5000414

[2] Pazianas M, Miller PD. Osteoporosis and chronic kidney disease–mineral and bone disorder (CKD-MBD): Back to basics. *American Journal of Kidney Diseases*. 2021;78(4):582-589. doi:10.1053/j.ajkd.2020.12.024

[3] Hernandez JD, Wesseling K, Pereira R, Gales B, Harrison R, Salusky IB. Technical approach to iliac crest biopsy. *Clinical Journal of the American Society of Nephrology*. 2008;3(Supplement 3). doi:10.2215/cjn.00460107

[4] Compston J.E. Bone histomorphometry. *Vitamin D*. 2005:951-965. doi:10.1016/b978-012252687-9/50062-0

[5] Christen P, Boutroy S, Ellouz R, Chapurlat R, van Rietbergen B. Least-detectable and age-related local in vivo bone remodelling assessed by time-lapse HR-pqct. *PLOS ONE*. 2018;13(1). doi:10.1371/journal.pone.0191369

[6] Nelson L, Gulenchyn KY, Atthey M, Webber CE. Is a fixed value for the least significant change appropriate? *J Clin Densitom*. 2010 Jan-Mar;13(1):18-23. doi: 10.1016/j.jocd.2009.10.001. PMID: 20171565.

[7] P. K. Saha and F. W. Wehrli, "Measurement of trabecular bone thickness in the limited resolution regime of in vivo MRI by fuzzy distance transform," in *IEEE Transactions on Medical Imaging*, vol. 23, no. 1, pp. 53-62, Jan. 2004, doi: 10.1109/TMI.2003.819925.

[8] Burghardt AJ, Pialat JB, Kazakia GJ, Boutroy S, Engelke K, Patsch JM, Valentinitsch A, Liu D, Szabo E, Bogado CE, Zanchetta MB, McKay HA, Shane E, Boyd SK, Bouxsein ML,

Chapurlat R, Khosla S, Majumdar S. Multicenter precision of cortical and trabecular bone quality measures assessed by high-resolution peripheral quantitative computed tomography. J Bone Miner Res. 2013 Mar;28(3):524-36. doi: 10.1002/jbmr.1795. PMID: 23074145; PMCID: PMC3577969.

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