Influence of reconstruction parameters of micro-computed tomography on the analysis of bone mineral density

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ABSTRACT

Purpose: This study was conducted to evaluate the influence of reconstruction parameters of micro-computed tomography (micro-CT) images on bone mineral density (BMD) analyses.

Materials and Methods: The sample consisted of micro-CT images of the maxillae of 5 Wistar rats, acquired using a SkyScan 1174 unit (Bruker, Kontich, Belgium). Each acquisition was reconstructed following the manufacturer’s recommendations (standard protocol; SP) for the application of artifact correction tools (beam hardening correction [BHC], 45%; smoothing filter, degree 2; and ring artifact correction [RAC], level 5). Additionally, images were reconstructed with 36 protocols combining different settings of artifact correction tools (P0 to P35). BMD analysis was performed for each reconstructed image. The BMD values obtained for each protocol were compared to those obtained using the SP through repeated-measures analysis of variance with the Dunnett post hoc test (α = 0.05).

Results: The BMD values obtained from all protocols that used a BHC of 45% did not significantly differ from those obtained using the SP (P > 0.05). The other protocols all yielded significantly different BMD values from the SP (P < 0.05). The smoothing and RAC tools did not affect BMD values.

Conclusion: BMD values measured on micro-CT images were influenced by the BHC level. Higher levels of BHC induced higher values of BMD. (Imaging Sci Dent 2020; 50: 153-9)

KEY WORDS: Artifacts; Bone Density; X-Ray Microtomography

Introduction

Dental implants have been widely used as a rehabilitation alternative in patients with missing teeth. Osseointegration of implants depends on factors related to the professional (e.g., experience and the choice of surgical approach), to the implant (e.g., macrostructure and microstructure), and to the patient (e.g., oral hygiene and the quantity and quality of host bone at the implant site).¹,²

Bone quality is crucial for the outcome of implant treatment.³ Bone mineral density (BMD), which is a factor that must be considered when assessing bone quality, can be evaluated through micro-computed tomography (micro-CT).⁴,⁵ Micro-CT is a non-invasive and non-destructive imaging modality that allows the visualization of structures at a micrometric level,⁶,⁷ providing image reconstructions with a high spatial resolution for detailed quantitative and qualitative analyses.⁷,⁸

Micro-CT is mostly used for scientific research purposes, due to its high radiation dose and the small image receptor.⁶ In implantology, it has been widely employed in studies on cortical thickness,⁹ the effects of age and osteoporosis,¹⁰ bone-implant integration,⁴ and bone characterization, such as bone volume fraction,⁴ bone microar-
chitecture, and bone quality. In the context of in vitro studies, along with histomorphometric analysis, micro-CT is considered to be a gold standard for bone density, as it provides information necessary for quantifying bone density, such as the ratio of bone volume to total volume. Additional advantages of using micro-CT rather than conducting a histomorphometric analysis include the possibility of a 3-dimensional microarchitectural analysis, less error due to shrinkage, greater time-effectiveness, and lower costs.

Due to the characteristics of the radiation beam and detector, there are image artifacts in micro-CT that can affect image quality, such as beam hardening, ring artifacts, and noise. To overcome this limitation, specialized tools may be used during image reconstruction to reduce or eliminate these artifacts, such as beam hardening correction (BHC), a smoothing filter, and ring artifact correction (RAC).

Attempting to correct beam hardening artifacts on micro-CT is important because these artifacts degrade the image quality with the appearance of cupping and streak lines, which may influence the linear attenuation coefficient values in the reconstruction of the image, consequently resulting in differences in measurements of BMD and bone mineral content. Although the manufacturer provides a protocol for these artifact correction tools, the manufacturer advises adjusting the intensity of these tools according to the clinician’s subjective judgment.

Therefore, the aim of the present study was to evaluate the influence of artifact correction tools in the reconstruction of micro-computed tomography images for the analysis of BMD.

Materials and Methods
Micro-CT acquisition of the study sample
The present study was exempted from review by the local Institutional Ethics Committee on Animal Use since the sample consisted of micro-CT images of the maxillae of 5 female Wistar rats that were acquired for previously approved research (protocol 3344-1/2014).

The micro-CT scans were performed on a Skyscan 1174 micro-CT unit (Bruker, Kontich, Belgium) using the following settings: 50 kV; 800 μA; voxel size, 10.2 μm; aluminum filter, 0.5 mm; rotation step, 0.5º; 2 frames per rotation degree; and 180º rotation. Each maxilla was fixed with wax on the scanning platform to ensure a standardized position with the long axis vertical to the horizontal plane and individually scanned while wrapped in wet paper to maintain moisture during the scanning procedure, which lasted 35 minutes. All methods related to the acquisition, reconstruction, and image analysis were conducted by an oral radiologist with 6 years of experience in micro-CT imaging.

Micro-CT evaluation: BMD analysis
BMD was assessed using CTAn version 1.17.7.2+ software (Bruker, Kontich, Belgium). Calibration was carried out before each BMD calculation, since the primary entity measured by micro-CT is the absorption of X-rays, defined as the attenuation coefficient per distance unit (mm), which in turn is determined by both the mass density and the elemental composition of the material. This calibration is done by scanning the material known to be the main substance responsible for the absorption...
of X-rays, making it possible to correlate the measured X-ray attenuation coefficient to the mass density of this material.18

Assuming that X-ray attenuation in mineralized bone tissues can be approximated as the X-ray attenuation of calcium hydroxyapatite,18 aqueous solutions of dipotas- sium phosphate (K2HPO4) were used to simulate the absorption of X-rays by the bone for software calibration. K2HPO4 is a highly water-soluble salt with an effective atomic number very close to that of calcium hydroxyapatite (15.58 and 15.86, respectively), 19 and has been previously used as a calibration phantom to measure attenuation coefficients in micro-CT.20

Thus, the software calibration was performed using a phantom composed of a solution of K2HPO4 at two different concentrations (600 and 1200 mg/mL), which produce attenuation coefficients equivalent to the trabecular bone and cortical bone, respectively.19-21 These solutions were placed in 2 Eppendorf tubes (0.2 mL; Axygen, Union City, CA, USA), which were positioned vertically and scanned together, using the same parameters as the study samples (Fig. 1). Subsequently, the phantom image was reconstructed using the protocols studied (SP and P0 to P35). The attenuation coefficient of both K2HPO4 solution concentrations was determined for each protocol to adjust the formula for BMD analysis. This step was done before the BMD calculation for each protocol.

After the formula adjustment, the reconstructed images of the rats’ maxillae were also imported into the CTAn software. A standardized circular region of interest (ROI) (consisting of 100 slices, 0.5 mm in diameter) in the fura- culation region of the right upper second molar was determined and saved, so that the same ROI could be applied in the analysis of all samples (Fig. 2). Threshold values were set for bone and the calibration phantom (maximum, 255; minimum, 61). Then, the BMD (g/cm3) for the ROI was obtained.

Statistical analysis
SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. The mean BMD values obtained using each protocol were compared to those obtained using the SP by repeated-measures analysis of variance (ANOVA) with the Dunnett post hoc test. The statistical significance level was set at P < 0.05.

Results
Table 1 shows the means and standard deviations of the BMD values obtained for all micro-CT reconstruction

| Protocols (BHC-SF-RAC) | BMD (g/cm³) | Protocols (BHC-SF-RAC) | BMD (g/cm³) |
|----------------------|------------|----------------------|------------|
| Standard protocol (45-2-5) | 1.60 ± 0.08 | P0 (0-0-0) | 1.42 ± 0.08* |
| P1 (15-2-5) | 1.51 ± 0.08* | P19 (45-2-10) | 1.60 ± 0.08 |
| P2 (15-2-10) | 1.51 ± 0.08* | P20 (45-2-15) | 1.60 ± 0.08 |
| P3 (15-2-15) | 1.51 ± 0.08* | P21 (45-4-5) | 1.60 ± 0.08 |
| P4 (15-4-5) | 1.51 ± 0.08* | P22 (45-4-10) | 1.60 ± 0.08 |
| P5 (15-4-10) | 1.51 ± 0.08* | P23 (45-4-15) | 1.60 ± 0.08 |
| P6 (15-4-15) | 1.51 ± 0.08* | P24 (45-6-5) | 1.60 ± 0.08 |
| P7 (15-6-5) | 1.51 ± 0.07* | P25 (45-6-10) | 1.60 ± 0.08 |
| P8 (15-6-10) | 1.51 ± 0.07* | P26 (45-6-15) | 1.60 ± 0.08 |
| P9 (15-6-15) | 1.51 ± 0.07* | P27 (60-2-5) | 1.63 ± 0.08* |
| P10 (30-2-5) | 1.56 ± 0.08* | P28 (60-2-10) | 1.63 ± 0.08* |
| P11 (30-2-10) | 1.56 ± 0.08* | P29 (60-2-15) | 1.63 ± 0.08* |
| P12 (30-2-15) | 1.56 ± 0.08* | P30 (60-4-5) | 1.62 ± 0.08* |
| P13 (30-4-5) | 1.64 ± 0.20 | P31 (60-4-10) | 1.62 ± 0.08* |
| P14 (30-4-10) | 1.56 ± 0.08* | P32 (60-4-15) | 1.71 ± 0.13* |
| P15 (30-4-15) | 1.56 ± 0.08* | P33 (60-6-5) | 1.62 ± 0.08* |
| P16 (30-6-5) | 1.60 ± 0.13 | P34 (60-6-10) | 1.62 ± 0.08* |
| P17 (30-6-10) | 1.56 ± 0.08* | P35 (60-6-15) | 1.62 ± 0.08* |

BHC: beam hardening correction, SF: smoothing filter, RAC: ring artifact correction, *: P < 0.05 compared with the standard protocol.
protocols. The BMD obtained using the SP was 1.6 ± 0.08 g/cm³, while using P0, a protocol without application of any artifact reduction tool, this value decreased to 1.4 ± 0.08 g/cm³. However, it was observed that the BMD values obtained using the protocols with BHC at 45% (P19 to P26) and with BHC at 30% (P13 and P16) did not significantly differ from those obtained using the SP (P > 0.05). For the other protocols, the BMD value was directly proportional to the degree of application of the BHC tool, yielding a statistically significant difference from the SP values (P < 0.05) (Fig. 3). In general, the BMD values obtained using protocols with a BHC at 15% were slightly above 1.5 g/cm³, while the protocols with BHC set at 30% resulted in BMD values between 1.5 and 1.6 g/cm³, those with BHC set at 45% generated BMD values of 1.6 g/cm³, and protocols with BHC set at 60% resulted in BMD values slightly above 1.6 g/cm³. Variation in the settings of the smoothing and RAC tools did not affect BMD values (Table 1).

**Discussion**

The results showed a direct relationship between measured BMD values and the BHC level (i.e., BMD values
were higher when protocols with higher BHC levels were used). Due to the polyenergetic characteristic of X-ray beams, beam hardening causes a discrepancy in the linear attenuation coefficient assumption. As an effect of this phenomenon, the edges (smaller thickness) of the scanned sample seem to be denser than the central region (higher thickness), also known as the “cupping” effect.

Beam filtration is an option for reducing beam hardening. Meganck et al. evaluated the influence of different thicknesses of aluminum and copper filters on beam hardening for BMD evaluation in murine bone, concluding that filtration added to a beam flattener did not require software processing for BHC, as shown by the lack of an impact on BMD estimation, although BHC may decrease image contrast and increase image noise. However, high filtration might impair the assessment of mouse bone and soft tissue.

Koubar et al. proposed a method for BHC based on linear attenuation curves for each material scanned (water, soft tissue, and bone), resulting in accurate bone mass measurements, with an error of ~3.4% for mouse skulls. A cone-beam micro-CT unit constructed at the researchers’ own institution was used in that study. The BHC tool in the NRecon software uses a linearization process based on a polynomial algorithm that reduces the non-linearity within the sample. As shown by Fajardo et al., as the level of BHC increases (in %), the mean attenuation of the sample also increases, resulting in a more hyperdense (brighter) image. This fact explains the direct relationship found between BMD and BHC levels in the present study. A previous study, despite the use of a different micro-CT unit, reported that BHC was the most important predictor for BMD. BMD errors related to the size of the samples are dependent on object structure and BHC level, with the latter being crucial for reducing these errors. The present study evaluated the impact of combinations of artifact correction tools in BMD values in comparison with the manufacturer’s protocol and found statistically significant differences. Further investigation of BHC levels in different samples regarding size, bone from different regions (e.g., jaw bones, long bones, etc.), different animals, and different micro-CT units should be carried out to establish optimum protocols for BMD assessment.

Ring artifacts may impair the quantitative 3D analysis of micro-CT data. Flat-field correction, detector shifting, and data inpainting can be pre-processing tools for minimizing ring artifacts, but do not completely remove these artifacts. Some post-processing methods have been reported successful for this task, using mean and medial filtering based on polar coordinates and an adaptive correction scheme. However, the value applied in NRecon (from 0 to 20) is determined empirically, and higher values are not recommended, as they can cause image blurring or even new ring artifacts. Noise can also impair 3D image analyses (e.g., edge detection, image segmentation, and volume rendering). Smoothing (also

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**Fig. 3.** Distribution graph showing the mean BMD values for each protocol tested. Different symbols represent the standard protocol (SP), the protocol without artifact correction tools (P0), and protocols with different degrees of beam-hardening correction (BHC), varying from 15% to 60%. BMD: bone mineral density, SF: smoothing filter, RAC: ring artifact correction.
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sometimes referred to as “blurring”) reduces noise by averaging the defective noise voxels with the gray values of neighboring voxels, normally by a Gaussian or median filter.

Despite the change in voxels’ gray values from both RAC and smoothing, which replace data by an average of surrounding values, those tools did not seem to influence the BMD measurements, except for P13 (BHC, 30%; RAC, 5; smoothing, 4) and P16 (BHC, 30%; RAC, 5; smoothing, 4) which unexpectedly did not differ from the SP. The standard deviations of protocols P13 and P16 were also higher than those of the other protocols, which may be attributed to this result. In contrast, P10 had the same RAC and BHC levels, but a lower smoothing of 2, and behaved as expected. Additionally, protocols P18 (BHC, 30%; high RAC; medium smoothing) and P32 (high BHC; high RAC; and medium smoothing) presented unexpected behavior, with higher BMD values than the other protocols with the same BHC. More studies should focus on understanding not only the behavior of these tools in isolation, but also their behavior when combined.

Gramanzini et al.5 concluded that micro-CT is a viable tool to characterize bone density. However, that study, as well as most previous studies that assessed BMD through micro-CT for bone quality assessments in implantology, did not report the reconstruction parameters and artifact correction tools that were applied,4,5,9,10,28 while Hua et al. did not report the reconstruction parameters and artifact correction tools for bone quality assessments in implantology, as well as most previous studies that assessed BMD through tool to characterize bone density. However, that study, as al.11 only cited the smoothing values used. According to our results, these reconstruction protocols influence BMD analysis, which makes it difficult to adequately analyze and compare studies. Therefore, the reconstruction parameters should always be described in future research.

Proper calibration is necessary to ensure the accuracy of BMD analysis. The present results were obtained using a home-made phantom with homogeneous solutions of K2HPO4 at 2 different concentrations (600 and 1200 mg/mL), with attenuation coefficients equivalent to the trabecular and cortical bone, respectively. K2HPO4, which is water-soluble, displays the same absorption characteristics as hydroxyapatite over a wide range of energies and overcomes the solubility problem of using the mineral hydroxyapatite, which is the main component of calcified bone that causes X-ray attenuation.29 The accuracy of the mineralization density of this compound was determined by comparing its measured linear attenuation coefficients with the theoretical values from previous studies.20,29,30 Furthermore, the recommendations of the micro-CT unit manufacturer regarding size, diameter, and positioning were followed when making the phantoms.18

In conclusion, this study showed that the level of the BHC tool used in the reconstruction of micro-CT images influenced the BMD analysis; specifically, higher BHC levels resulted in higher BMD values. Therefore, direct comparisons between studies using different BHC levels are not appropriate. Calibration of the scanned sample density and dimensions, with adequate phantoms, is recommended to ensure reproducibility. A single micro-CT unit was used in this study, so further research is required to evaluate how other micro-CT units and their artifact correction tools influence the values obtained in BMD analysis.

Conflicts of Interest: None

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