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Computer modelling integrated with micro-CT and material testing provides additional insight to evaluate bone treatments: Application to a beta-glycan derived whey protein mice model

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ABSTRACT

The primary aim of this study was to evaluate the influence of a whey protein diet on computationally predicted mechanical strength of murine bones in both trabecular and cortical regions of the femur. There was no significant influence on mechanical strength in cortical bone observed with increasing whey protein treatment, consistent with cortical tissue mineral density (TMD) and bone volume changes observed. Trabecular bone showed a significant decline in strength with increasing whey protein treatment when nanoindentation derived Young's moduli were used in the model. When micro-indentation, micro-CT phantom density or normalised Young's moduli were included in the model a non-significant decline in strength was exhibited. These results for trabecular bone were consistent with both trabecular bone mineral density (BMD) and micro-CT indices obtained independently. The secondary aim of this study was to characterise the influence of different sources of Young's moduli on computational prediction. This study aimed to quantify the predicted mechanical strength in 3D from these sources and evaluate if trends and conclusions remained consistent. For cortical bone, predicted mechanical strength behaviour was consistent across all sources of Young's moduli. There was no difference in treatment trend observed when Young's moduli were normalised. In contrast, trabecular strength due to whey protein treatment significantly reduced when material properties from nanoindentation were introduced. Other material property sources were not significant but emphasised the strength trend over normalised material properties. This shows strength at the trabecular level was attributed to both changes in bone architecture and material properties.

1. Introduction

Micro-finite element (FE) techniques based on high resolution images allow for the modelling of trabecular and cortical bone in detail. This process uses images from micro-CT scans and converts voxels into elements to produce meshes suitable for FE analysis [1,2]. Computational modelling is able to non-destructively and rapidly evaluate the quality of bone in response to various treatments. This is advantageous as it is less costly, can reduce sample numbers in large clinical studies and leaves the bone available for future histological and mechanical testing. Computational modelling has been shown to achieve precision comparable to densitometry data and is able to predict fracture loads up to an accuracy of 60% [3]. Additionally, destructive compressive tests on the radius have shown strong correlations with equivalent FE models [4]. There have been studies that have used micro-FE models to assess bone strength in response to treatments [5–7]. Those studies revealed that modelling isolates strength changes in cortical and trabecular bone separately, that subject-specific variations within groups are important and that modelling reveals whole strength measures that may be different from surrogate measures

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of strength (like micro-CT indices). The current study demonstrates all 3 aspects.

While FE modelling has been shown to play a useful role in evaluating bone, there are few studies in the literature that compare computer predictions of bone strength against experiment using a double blinded design. In this study we evaluate computational predictions of strength against traditional micro-CT surrogates and see if the conclusions drawn are consistent. Secondly, while it is known that material properties derived from different sources and scales produce different Young's modulus estimates, it is of interest to know how much this influences computational predictions and conclusions drawn as a result.

At the macro-scale, bone is simply divided into cortical and trabecular bone. Cortical bone is the hard, outer layer whereas trabecular bone is porous and predominantly fills the ends of long bones. Fundamentally, trabecular and cortical bone are made up of the same constituents; however mechanically, the homogenised mechanical response of trabecular and cortical bone vary greatly due to their site specific architecture and material properties. Homogenised Young's modulus of cortical bone can be anywhere from 14 to 29 GPa [8,9], while homogenised Young's modulus of trabecular can be 100 MPa to 4 GPa [9,10]. Mechanical properties of bone are determined not only by bone mineral composition but also the structural organisation at different sub scales (microstructural and nanostructural). Therefore, measurement of material properties at different scales incorporates different levels of porosity and homogenised underlying structure. Within bone tissue exists a complex hierarchical system. In humans, the Haversian systems, osteons and individual trabeculae are found in the microstructural layer, which contribute to homogenised material properties at the macro-level [11]. It should be noted that while human bone consists of a Haversian system, mice lack one [12]. The lamellae are found in the sub-microstructural layer. At the nanostructural level is where fibrillar collagen and embedded minerals are found and the sub-nanostructure contains the molecular structure such as the collagenous and non-collagenous organic proteins [8,13]. These structures contribute to properties measured through nanoindentation. Microindentation and nanoindentation tests are used to determine the hardness of bone tissue at different structural levels [14], which are used to inform micro-FE models. Three point bending tests can also provide an indication of the axial mechanical strength of cortical bone [15]. As treatment effects manifest at different levels of bone, using material properties derived from different scales may highlight the treatment effect that may otherwise be hidden.

The mechanical properties of bone are dependent on several diverse factors which can include but are not limited to age, diet, exercise and disease [16]. Bone can also be affected by treatments differently in trabecular and cortical zones. This is the reason why computational models that isolate cortical and trabecular behaviour provide insight into compartmentalised bone response. It is also known that bone strength is dominated by the cortical response but still needs trabecular support to prevent internal buckling. Endocortical thinning is usually the last stage of bone degradation, following trabecular resorption [17], hence, while net bone strength may not be compromised, evaluation of trabecular bone strength is an early indicator of future mechanical failure.

In order to test the use of computational modelling as an informative tool in bone treatment studies, we conducted a variable whey protein controlled murine study. Twenty nine mice were fed a varied cheese diet (with whey protein), later euthanised and their femurs obtained for testing. This study integrated micro-architectural indices from micro-CT, mechanically derived material properties and micro-FE modelling. Specifically, the objectives of this study were to determine (i) whether computer modelling can predict trends in treatment outcomes locally within cortical and trabecular bone by assessing the geometrical architecture alone (with normalised material properties) in a double blinded design; and (ii) are computational predictions of strength across treatments consistent when material properties are normalised and derived from nanoindentation, microindentation, three point bending and micro-CT phantom derived grey scale values.

2. Materials and methods

12 week old, female, C3H mice were divided into three groups: placebo = 9, 0.1% whey protein = 9 and 1% whey protein = 11. Animals were fed to the point of sacrifice with either the control diet (AIM-93M including 180 g of soy protein per kg), or a diet in which 10 g of soy was replaced by 0.1% or 1% of beta-glycan derived whey-protein. At 26 weeks, the mice were anaesthetised with isoflurane and morphine was administered to avoid pain. Whole blood was collected by cardiac puncture, mice were then euthanised and femurs were excised, cleaned of the surrounding soft tissue and fixed in 70% ethanol. The distal end of the femurs was scanned using a SkyScan 1172 micro-CT scanner (X-ray voltage 50 kV, 0.5 mm aluminium filter, voxel size 5 μm). Standardised reconstructions were carried out using SkyScan NRecon software and the datasets were analyed using SkyScan micro-CT analyser software, CTAn (version 1.14.4.1). Fig. 1 shows where the volumes of interest (VOIs) of trabecular and cortical bone were selected from: the trabecular region of bone was 0.4 mm proximal to the growth plate and extended 1.5 mm in the proximal direction and the cortical region of bone was 3.25 mm proximal to the growth plate and extended 0.5 mm in the proximal direction. BMD and TMD values were obtained from calcium hydroxyapatite micro-CT phantoms and were averaged for the VOI. Each dataset was binarised using global thresholding (90–255). Using CTAn, 3D micro-CT indices were measured which included percentage bone volume (BV/TV), trabecular thickness (TbTh), trabecular separation (TbSp) and trabecular number (TbN) for trabecular bone; and mean total cross sectional tissue area (T.Ar), mean total cross sectional bone area (B.Ar), mean medullary area (Ma.Ar), mean cortical area fraction (B.Ar/T.Ar) and mean cross sectional thickness (Cs.Th) for cortical bone for all 29 femur samples.

The raw digital data in StereoLithography (STL) format was digitally cleaned and this process removed any bone fragments (any part of the bone not attached to the main body) and redundant polygons. The mesh was decimated (reduced in size) for computational efficiency without affecting the overall quality of the bone geometry, using Rapid Form (Inus Technology Inc.). The solution time to fit a FE voxel mesh to the STL triangle mesh was dependent on the number of triangles present. The process was optimised by finding the minimum density of triangles in the STL mesh so that the resulting voxel mesh varied by less than 0.1% when computing strain compared to the original mesh density. A reduction of 30% in the triangle number did not affect bone geometry difference by more than 0.02 mm. Following from this, a hexahedral voxel mesh was created for each bone sample using a mesh generation package, Hypermesh (Altair Engineering Inc.). Voxel cubes were chosen as they have a robust and stable behaviour for elastic mechanics compared to tetrahedral meshes in FE simulations. On average, trabecular bone had between 300,000 and 400,000 elements and cortical bone had between 11,000 and 13,000 elements. Aspects of this framework are shown in Fig. 2.

A previous validation study (see Fig. 3) using a 3D printed trabecular bone model with a Young's modulus of 1.4 GPa and Poisson's ratio of 0.3 was subjected to an Instron compression test and subsequently, replicating the Instron test as an FE simulation resulted in a von Mises failure criteria for the bone models. Three