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Three-dimensional microarchitecture of enthesal changes: preliminary study of human radial tuberosity

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ABSTRACT Enthesal changes (EC), alterations at insertion sites on the bones, may be related to mechanical stress among other causes, and are thus used for decades to reconstruct the activities of human past populations. They can be characterised by focal changes in robusticity and variable pattern of osteolysis and osteoformation observable on dry bone. This preliminary study aims to analyse the microstructural characteristics of the underlying bone to clarify the nature of the changes in bone microarchitecture reflecting the macroscopic changes identifiable on the surface of the enthesis. We studied the right *radii* of two Saharian adult individuals, dating from Neolithic (Hassi-el-Abiod, Mali, 7 000 years BP). One has a morphologically normal bicipital tuberosity while the second one shows EC. Micro-computed tomodensitometric acquisitions and 3D reconstructions were performed to characterise cancellous and cortical bone microarchitecture of these two entheses. 3D imaging appears relevant for studying microstructure of EC among past populations. Our methodology reveals at this preliminary step clear differences of canal network organisation of cortical bone between the two studied entheses. This work comes preliminary to a broader study on a historically and archaeologically documented population of Hungarian horse archers of the Honfoglalás or Conquest period (Xth century).

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KEY WORDS

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Introduction

Entheses are the insertion sites of tendons, ligaments and joint capsules on the bone. A distinction can be made between two types of entheses, fibrous entheses mainly at the metaphyseal or diaphyseal areas, and fibrocartilaginous entheses, including the insertions at the epiphyses and processes of long bones but also the short bones of hands and feet as well as several ligaments in the spine (Benjamin and Ralphs 1998; Benjamin and McGonagle 2001).

Among enthesal changes (EC), enthesopathies are defined by pathological modifications at the insertion sites (La Cava 1959; Niepel and Sit'aj 1979; Lagier 1991; Benjamin et al. 2002). They result in bone alterations visually observable on dry bone. It takes the form of osteophytic formations on the margin of the enthesis and/or an erosive area (porosity/

foramina/cysts) on its surface (eg. Hawkey and Merbs 1995; Robb 1998; Mariotti et al. 2004; Villotte 2009).

Enthesal changes can be due to several causes and can be related to age (that is strongly correlated with EC), to sex or some metabolic or inflammatory diseases, but these changes may also be caused by mechanical stress (e.g. Dutour 1992; Claudepierre and Voisin 2005; Slobodin et al. 2007; Villotte and Kacki 2009; Jurmain and Villotte 2010; Paja et al. 2010; Jurmain et al. 2012; Milella et al. 2012; Alves Cardoso and Henderson 2013; Henderson and Alves Cardoso 2013; Niinimäki and Baiges Sotos 2013; Niinimäki et al. 2013; Villotte and Knüsel 2013; Santana Cabrera et al. 2015). Indeed, one of the fundamental roles of an enthesis is stress dissipation, distributing load forces across the bone (Benjamin and McGonagle 2001). Therefore, EC can in certain conditions indicate an intense muscle solicitation during life, and so have been considered for decades as occupational stress markers, with the perspective of reconstructing activities and lifestyles of ancient populations (eg. Dutour 1986; Hawkey and Merbs 1995; Pálfi 1997; Peterson 1998; Molnar 2006; Rojas-

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Sepúlveda and Dutour 2009; Villotte et al. 2010b; Baker et al. 2012). This question is fundamental in the field of bioarchaeology and is even considered by some scholars as a sort of “Holy Grail”, as Jurmain et al. (2012) pointed out.

Entheses and their changes have been extensively studied, with clinical, radiological, histological and osteological methods at the macroscopic and microscopic scales (e.g. Cooper and Misol 1970; Resnick and Niwayama 1983; Olivieri et al. 1998; Benjamin et al. 2002; Claudepierre and Voisin 2005; Maffulli et al. 2005; Benjamin et al. 2008; Villotte 2009; Junno et al. 2011; Schlecht 2012; Henderson 2013a). Recently, the 3D approach has begun to be used as a tool for studying the EC, but researches focus only on enthesal surfaces (Pany et al. 2009; Henderson 2013b; Noldner and Edgar 2013; Nolte and Wilczak 2013). We chose here the complementary use of micro-tomodensitometry investigation and 3D reconstructions, which have been little applied to occupational markers so far: in their study Djukic et al. (2015) used micro-tomodensitometry for performing metric analyses of bone microarchitecture on several lower limb entheses, to study the relationship between macro- and microstructure, as well as between cortical and trabecular parameters at enthesal level. They showed a lack of correlation between macroscopic scoring systems with microarchitecture at the entheses.

Our preliminary study takes its part in the framework of a broader one, which will focus on bioanthropology and archaeology of mounted archers of the Hungarian Conquest period, so called Honfoglalás (Xth century AD). Our aim is to explore bone microarchitecture of a morphologically normal insertion and EC at the radial tuberosity, hypothetically likely to be related to repeated movement involved in archery, among other activities (Dutour 1986; Thomas 2014; Tihanyi et al. 2015) in order to present the observations that can be performed this way. In the long run, our purpose will be to specify the microstructural changes in the underlying bone that reflect the surface bone changes resulting from mechanical stress. The ability to distinguish, in a reliable way, mechanical enthesopathies from EC resulting from other aetiologies, would allow us to bring new elements to questions as: who were mounted archers? Did they belong to a special class among the population? At what age did they begin training for war? Did the women practiced horseback riding and archery as well?

Furthermore, this study constitutes a first test of the application of the methodology to EC, which we will employ for larger investigations. We performed bone microarchitectural analyses at entheses sites, with the help of microtomodensitometry and 3D imaging (Coqueugniot et al. 2010; Colombo 2014). The goal of this paper is to open up the field of occupational marker studies to innovative methodological approaches such as bone microstructural analyses.

Materials and Methods

We focused here on the radial tuberosity, which is the insertion site of *biceps brachii*, and one of the fibrocartilaginous entheses (Benjamin et al. 1986). These are the most documented group of entheses in the attempt to reconstruct past activities (Havelková and Villotte 2007; Villotte 2009; Villotte et al. 2010a; Weiss 2012; Henderson et al. 2013; Villotte and Knüsel 2013; Thomas 2014). The *biceps brachii* is one of the flexor and supinator muscles of the elbow, and its changes at insertion site on the radius were previously interpreted to be linked with occupation (Dutour 1986; Hawkey and Merbs 1995; Pálfi 1997; Robb 1998; Molnar 2006; Weiss 2007; Baker et al. 2012; Thomas 2014; Tihanyi et al. 2015). For example, agricultural and building activities, and especially carrying heavy loads, have revealed to potentially cause EC at the bicipital tuberosity (Commandré 1977; Galera and Garralda 1993; Al-Oumaoui et al. 2004; Havelková et al. 2011; Rojas-Sepúlveda and Dutour 2014).

We relied on two adult individuals from Hassi-el-Abiod (northern Mali), belonging to a Neolithic population of hunters-fishermen-gatherers who lived close to lake or wetland areas about 7 000 BP. The anthropological study performed by one of us (OD) in 1986 had already identified EC on upper limbs in this population (Dutour 1986, 1989).

Both right *radii* of those subjects have been considered (Fig. 1) and described with the terminology used in the Coimbra method (Henderson et al. 2013). Nevertheless, considering the weak number of bones, the scoring of each feature did not seem pertinent. The first one (MN10/H3) shows a normal, regular and smooth entheses, without any trace of bone formation or erosion at the margin and enthesal surface, nor porosity or cavitation. The second one (MN36/H18) presents changes at the insertion level: the margin is irregular and characterised by a sharp crest (medial and proximal), heightened by a marginal enthesophyte, while the surface is also irregular, with erosion on more than half the surface and a little macro-porosity.

In order to investigate bone microarchitecture of the entheses, we used microtomodensitometry, which provides, in a non-destructive way, information on the biomechanical properties of bone and the characteristics of bone remodelling through a three-dimensional approach (eg. Lespesailles et al. 2006; Colombo 2014; Rittemard et al. 2014; Khoury et al. 2015).

We applied the micro-computed tomography (micro-CT) acquisitions processing chain (Coqueugniot et al. 2011) developed in research unit PACEA (UMR 5199, CNRS/University of Bordeaux, Pessac, France), including image processing with TIVMI[®] (Treatment and Increased Vision for Medical Imaging) software. It is based on the HMH (Half



Figure 1. Bicipital tuberosity on the two selected right *radii*. Normal entheses presents smooth and regular surface and margin (left) and other one exhibits irregularities, porotic alterations and enthesophytes (right).

Maximum Height) 3D algorithm, which allows the software to automatically identify the optimal limits between each material such as bone and air (Spoor et al. 1993; Dutailly et al. 2009). The *radii* were CT scanned at PLACAMAT (UMS 3626, CNRS/University of Bordeaux, Pessac, France), on a GE® Phoenix v|tomelx s, with an isotropic resolution of 13.5 μm . We focused the acquisitions on the entheses area. The micro-CT was operated at 120 kV and 110 μA , with a 500 ms integration time per projection.

The data, which are slices in the three plans of space, were then treated with TIVMI® software to obtain 3D reconstructions from their superposition.

Several preliminary steps were required in order to analyse the microarchitecture of the entheses. We realised a primary 3D reconstruction of the whole entheses in order to globally visualise enthesal surface for selecting regions of interest (ROIs), on which observations were then performed (Fig. 2).

In both *radii*, we selected a portion localised in the proximal third of the enthesis, whose height was about 10% of the total entheses height, which was visually estimated regarding the superior and inferior portions of the margin (clearly observable for both entheses) and considered in terms of number of horizontal slices between these limits. It corresponds to bounding boxes with dimensions of 9.6 x 12.3 x 2 mm for the normal entheses and 7.3 x 13.2 x 2 mm for the other one, located from 23 to 33% of the entheses height, starting from the proximal margin. In addition, the ROIs were selected on the medial half of the tuberosity, where *biceps brachii*'s tendon does attach to the bone. We also ensured that these ROIs were long enough to catch a portion on the outside of the entheses, in order to investigate the transition between normal diaphyseal bone (on the medial-posterior face of the bone) and the entheses. We selected a second zone of interest in the case showing EC, at the upper margin of the entheses,

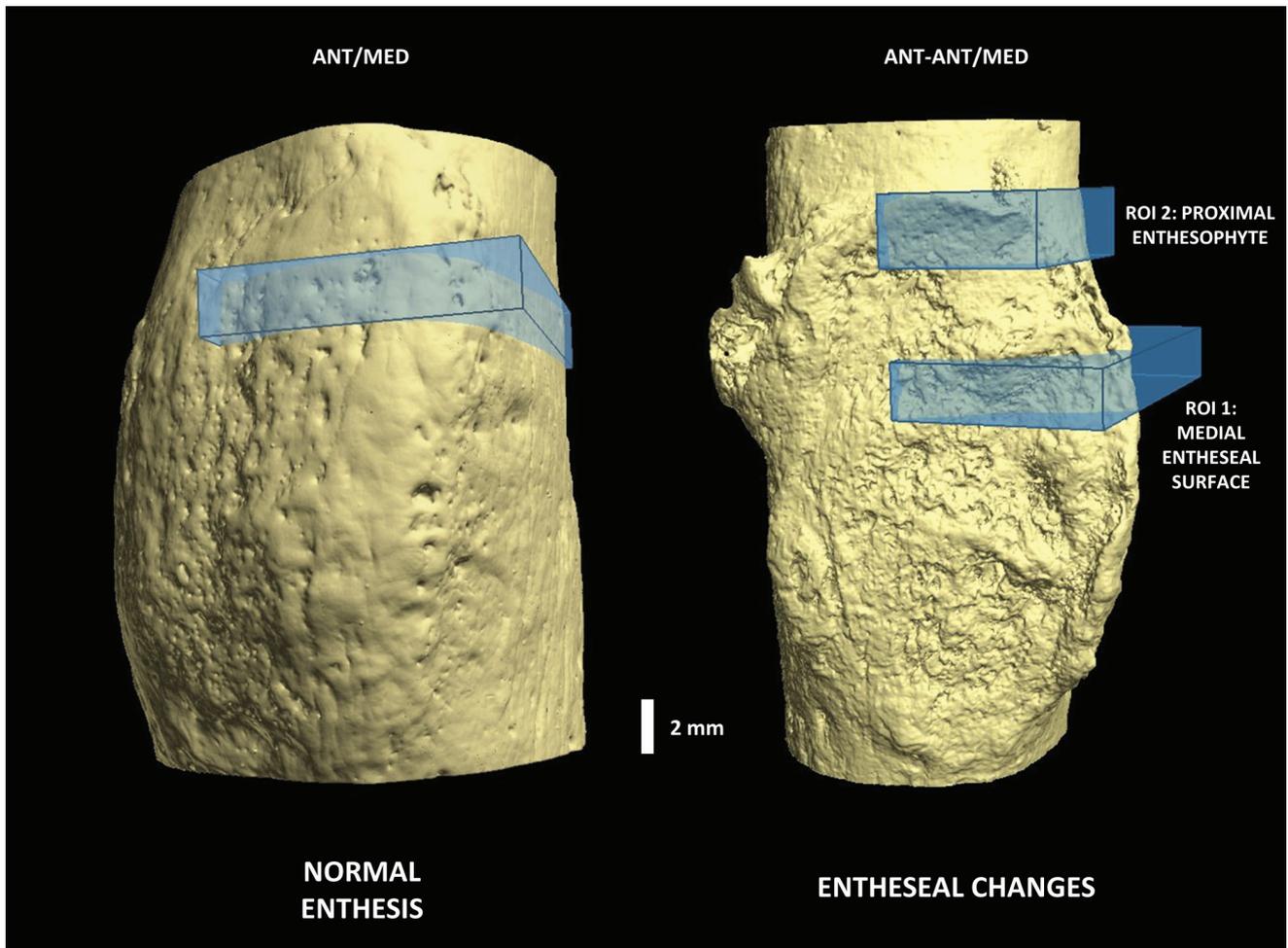


Figure 2. 3D reconstructions of both entheses showing the regions of interest (ROIs). Bounding boxes of the selected regions of interest appear in blue on the reconstructions. For the enthesis with changes, two levels of analysis were selected, one on the enthesal surface and medial margin (ROI 1), and the other at the proximal margin, focusing on the enthesophytic crest (ROI 2).

focusing on an enthesophyte. The corresponding bounding box dimensions are 5.5 x 8.4 x 2.4 mm and its height is about 12.5% of the total enthesis height.

We then operated a segmentation according to the grey level values of each component. It consists in the definition of subsets or materials, so the software is able to distinguish bone from empty canals and medullary cavities, external vacuum and sedimentary residues such as sand. In this case, due to the proximity of grey level values between bone and sediment, segmentation was manually performed. Subsequently, a binary image was obtained thanks to a double threshold. It consists of white pixels (the elements we want to keep in the 3D reconstruction) and black pixels (the elements to exclude). Finally, using a HMM algorithm, binary slices were superposed to reconstruct in three dimensions either

the bone or, on the contrary, the canal system of the cortical bone and the intertrabecular spaces of the cancellous bone, to observe their three-dimensional organisation.

This methodology, using micro-CT and 3D reconstructions with TIVMI® software program, was already performed in a recent research focusing on trabecular bone microarchitecture during growth, with good repeatability (Colombo 2014).

Results

We performed a visual morphoscopic analysis from these 3D reconstructed regions of interest.



Figure 3. 3D bone reconstruction of ROI at normal enthesis. It highlights the “trabecular aspect” of the enthesal volume and consequently the relative thinning of the cortical bone at the level of the normal tuberosity.

3D micro-architecture of normal enthesis

First, we observed in 3D reconstruction of the bone at entheses, the main characteristics of the normal structure, notably described from histologic sections (Benjamin et al. 1986; Claudepierre and Voisin 2005). The volume of the enthesis was limited by the endosteal wall of the medullary canal of the shaft (Fig. 3). Medullary cavities occupied by bone marrow and vessels were irregular in shape and size and were interconnected. Moreover, we observed that the enthesis mainly consists of trabecular bone while the cortical bone turns out very thin. Bone marrow extensions and thinning of cortical bone at entheses have been previously described in literature (Benjamin et al. 2002; Flemming et al. 2003; Benjamin et al. 2007; Shaw and Benjamin 2007; Nojiri et al. 2009).

Second, as regards the 3D organisation of the canal network of compact bone, three distinct areas were observable (Fig. 4):

1) outside the enthesis, on the medial-posterior face of the diaphysis, we observed a normal haversian organisation, with thin and vertical Havers’ and transversal Volkmann’s canals.

2) at the medial edge, we observed a horizontalisation of peripheral canals, while the canals on the inner edge of compact bone tend to preserve their longitudinal direction. Some canals were also thicker, in both directions.

3) on the anterior face of the enthesis, we noticed that the canals were differently organised, showing regular oblique interconnections. The global morphology could be described as a “trellis” (or lattice) aspect.

3D micro-architecture of enthesal changes

Regarding the first ROI, located on the medial enthesal surface and margin, similar observations to normal enthesis were

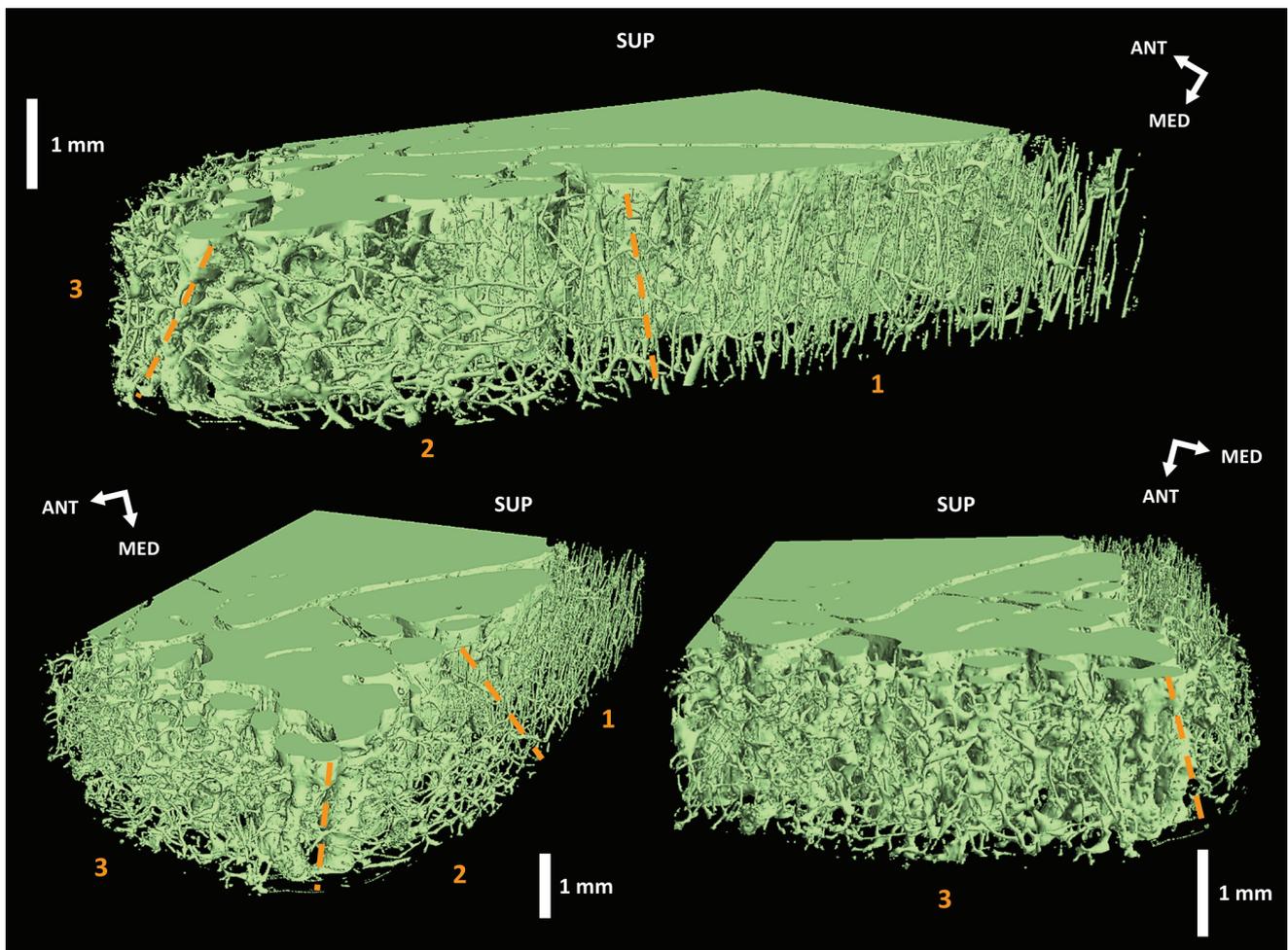


Figure 4. 3D canal network reconstruction of ROI at normal entheses. Three areas are observable: 1) normal Haversian organization; 2) horizontalisation and thickening of vascular canals; 3) “trellis” aspect of the canal organization.

performed on 3D bone reconstruction (Fig. 5). Medullary cavities communicated with the medullary canal of the diaphysis and had extended towards the exterior. In contrast, the cortical bone appeared even thinner than the normal aspect. This thinning was particularly pronounced at some places. The external entheses surface was irregular and it seemed that a few porosities were in connection with the underlying medullary cavities expanded inside the entheses volume.

The 3D reconstruction of the canal network showed similarities but also differences with the normal entheses (Fig. 6). We observed thin and longitudinal canals on the medial-posterior face of the diaphysis. Then, at the medial edge of the entheses, fewer canals were observable. They were not as longitudinally orientated as at the diaphysis area. Furthermore, the horizontal orientation observable at the area 2 of the normal entheses could not be observed here.

Moreover, at the anterior face of the entheses, the “trellis”

organization of the canals was not present either. Instead, it seemed that we had a non-organized bone, with thick and flattened canals.

Regarding the second ROI (Fig. 7), located at the proximal enthesophyte zone, we observed an apparent invasion of the compact bone area by spaces which seemed in relation to the medullary cavities. The trabecular bone was also organized in rows, parallel to the surface of the entheses.

Concerning the characteristics of the canals of cortical bone (Fig. 7), we observed Haversian canals on the medial-posterior face of the diaphysis and on the anterior face too, just above the origin of the enthesophyte. This one respected neither the Haversian organization nor the “trellis” organization. The canals were thick, flattened in the antero-posterior plan and the enthesophyte could thus be described as a non-organized bone production. It pleads for a primary ossification type, confirming that the marginal relief delineating the

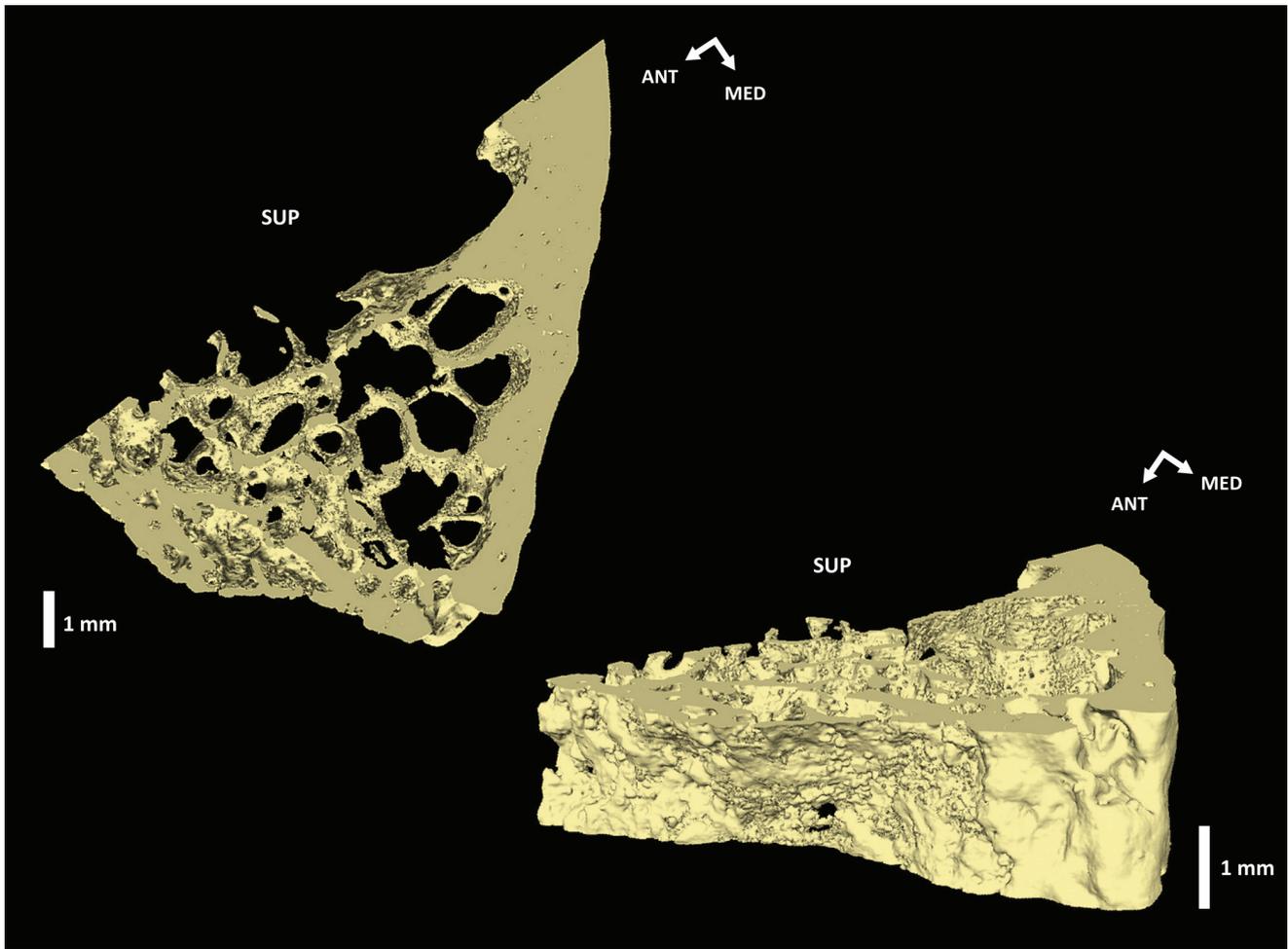


Figure 5. 3D bone reconstruction of ROI at EC surface. Expansion of trabecular organisation in the enthesal volume. Extreme thinning of the cortical bone at the level of the EC surface. Some porosities appear to be connected with medullary cavities.

eroded enthesal surface is not only due to erosion but to enthesophytic production. The global mechanism of enthesophyte ossification was described in literature: Benjamin et al. (2006) and Shaw and Benjamin (2007) made difference between tendon calcification and endochondral ossification, highlighting through vascularisation the possible role of bone marrow in the ossification process. In our case, medullar cavities are observed at the enthesophyte origin.

Discussion

As results of this preliminary study, we notice a different canal organisation at the enthesis with EC (both on the medial surface and the enthesophyte) from the normal enthesis. The aim of the present paper is not to validate the hypothesis of a mechanical origin linked to intense muscle load, but to present

the results of the first observations of microarchitecture of EC at the radial tuberosity, in comparison with a normal enthesis. What we have obtained here, with this exploratory approach, constitutes a promising preparatory work for larger studies, which will allow us to multiply the observations, and to test and validate our methodology. We expect to observe common and recurring features on a large number of normal insertions and enthesal changes. To do so, we will select samples taking account of the numerous biases inherent in studies aiming to reconstruct activities in ancient populations (e.g. Dutour 1992, 2000; Villotte 2009; Meyer et al. 2011; Jurmain et al. 2012; Milella et al. 2012; Schrader 2012; Alves Cardoso and Henderson 2013; Perréard Lopreno et al. 2013; Thomas 2014). In order to make comparisons with more objective criteria, we will also perform a skeletonisation method on smaller regions of the 3D reconstructions. It consists in making an object thinner (1 voxel wide) to keep its basic structure. It is based

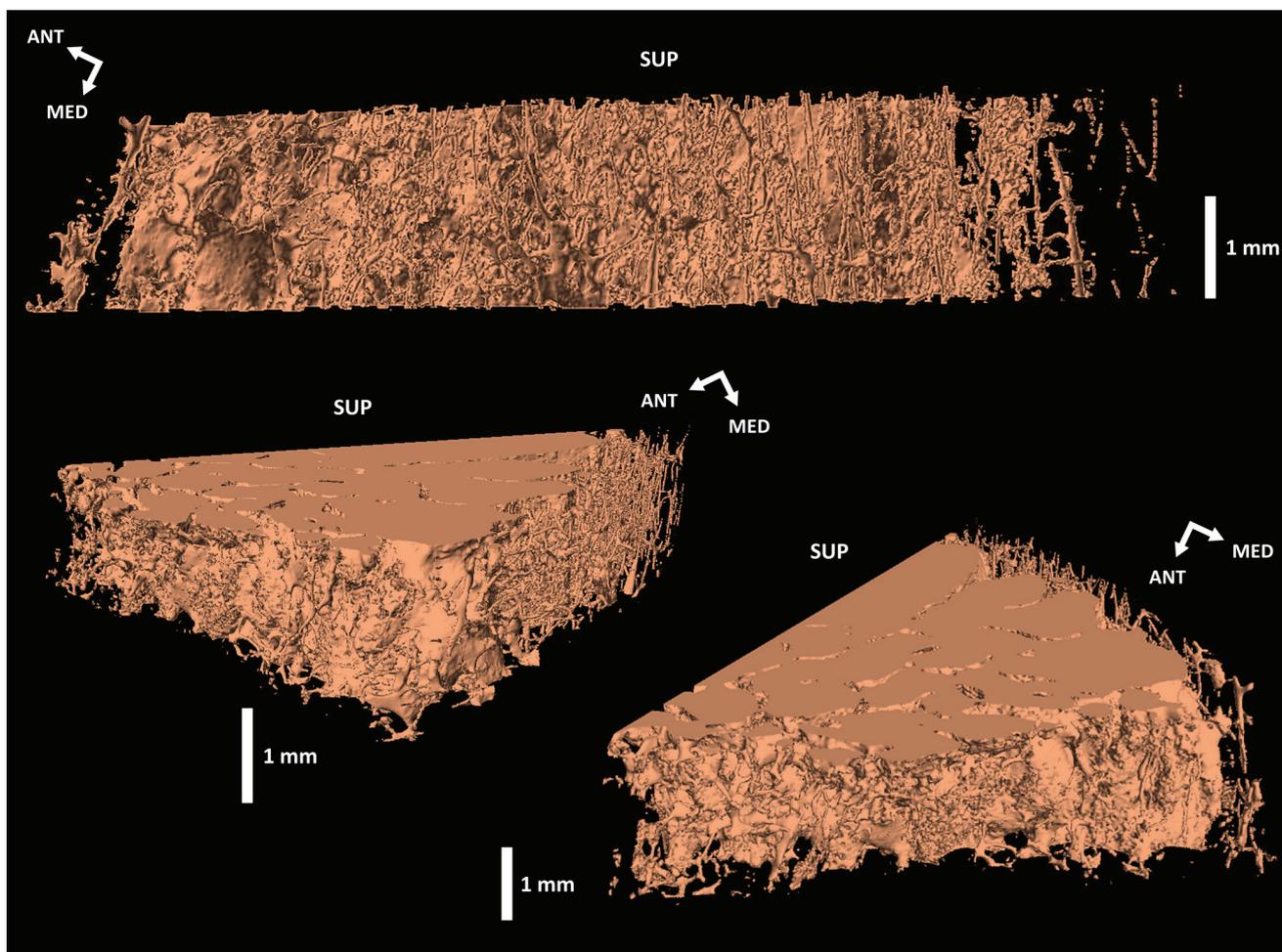


Figure 6. 3D canal network reconstruction of ROI at EC surface. The horizontalisation of the canals in cortical bone cannot be observed in this case, and the “trellis” organisation at the anterior face of the enthesis either. Instead, the vascular pattern appears non-organised.

on the sequential 3D curve-thinning algorithm developed by Palágyi et al. (2001) and implemented in TIVMI® software program. We will this way obtain a simplified modelling of the cortical microstructure allowing to determine qualitative and quantitative parameters (Colombo 2014). Once the method is validated for the study of EC in particular, we will be able to perform analyses on archaeological Hungarian material, consisting of horse archers of the tenth century, the Honfoglalás period. They left numerous cemeteries across the Carpathian Basin, where we can often discover archery-related items (such as bows, arrows and quivers) and even sometimes the horses in association with the skeletons within the graves (e.g. Révész 1996, 2003; Kovács 2005; Langó 2005; Révész 2005; Langó et al. 2011; Bíró 2014; Révész 2014). This enables us to put enthesal changes in relation to the archaeological goods, which is a decisive condition to discuss the activities of ancient populations (Thomas 2014). Hungarian skeletons have previously demonstrated their po-

tential for this type of studies (Pap 1985; Józsa et al. 1991; Pálfi 1992; Pálfi and Dutour 1996; Pálfi et al. 1996; Józsa et al. 2004; Tihanyi et al. 2015). This is a population of major interest, being well documented, and one of the most pertinent for methodological work on archaeological collections, especially for lifestyle reconstruction studies.

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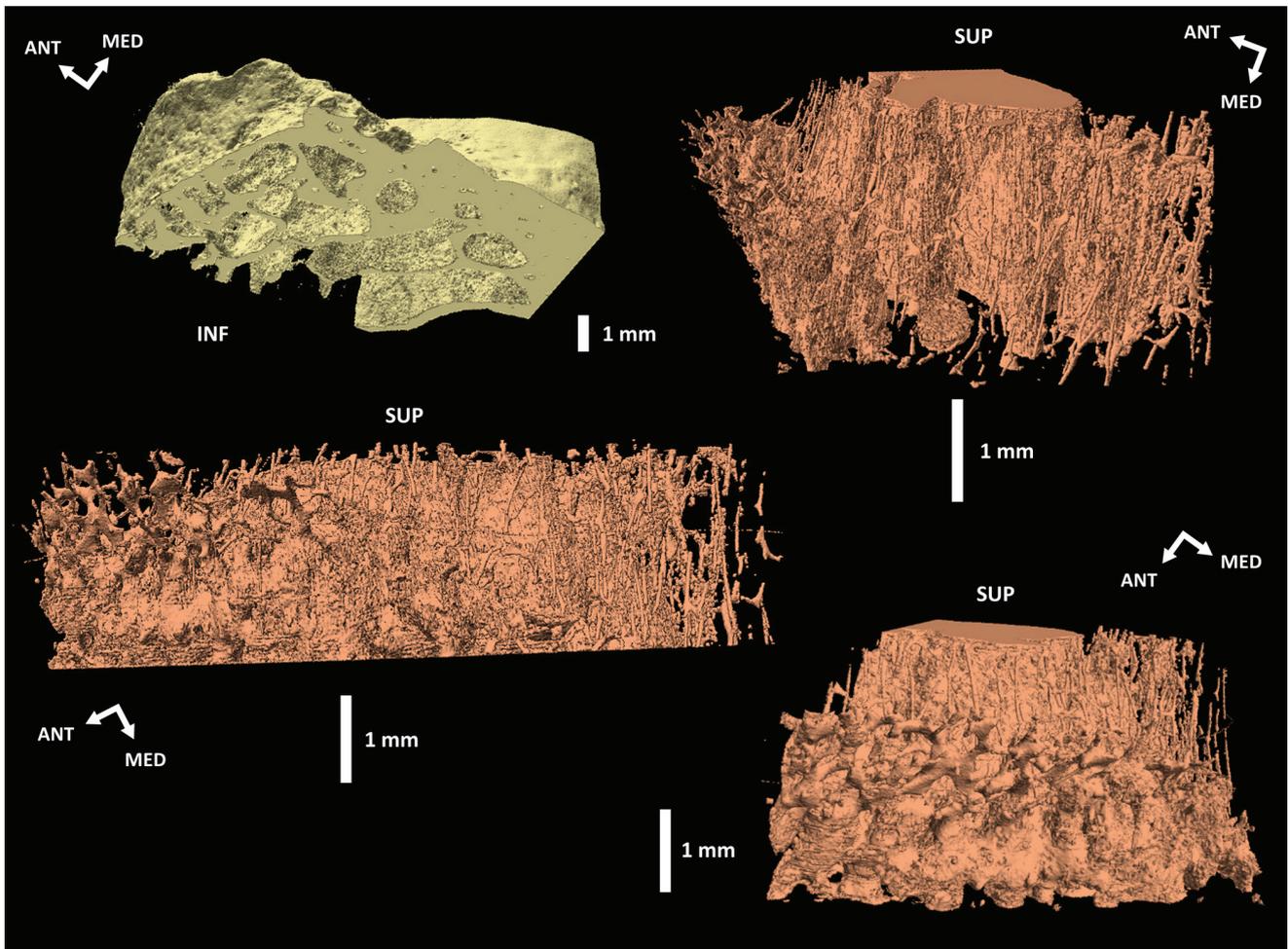


Figure 7. 3D bone and canal network reconstructions of ROI at enthesophyte level. One can observe a medullary invasion of the enthesophyte relief and irregular pattern of vascular canals, at the anterior face, in favour of a primary ossification.

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