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XI

THE TALKING DEAD

NEW RESULTS FROM CENTRAL AND EASTERN EUROPEAN OSTEOARCHAEOLOGY

TÂRGU MUREȘ | MAROSVÁSÁRHELY | 2016

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Editor

SZILÁRD SÁNDOR GÁL

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In memoriam Török Aurél
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CAN MICRO-CT AND 3D IMAGING ALLOW DIFFERENTIATING THE MAIN AETOLOGIES OF ENTHESEAL CHANGES?

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Keywords: Paleopathology; entheseal changes; 3D imaging; activity markers; radial tuberosity.

Abstract
Entheseal changes (EC), alterations at insertion sites on the bones, may be related to various causes, including mechanical stress and metabolic disorders such as Forestier's disease (DISH). This preliminary study aims to explore the osseous microarchitecture of the radial tuberosity to identify ECon the basis of their probable aetiology.
We relied on radii belonging to three male adults: (i) one from the Hungarian cemetery of Sárrétudvari-Hízóföld, probably a mounted archer from the Conquest period (Xth century), exhibiting EC; (ii) one from the Hungarian cemetery of Bácsalmás-Homokbánya (XVI–XVIIth centuries), showing EC associated with a DISH condition; (iii) one from the medieval cemetery of Val-de-Reuil (France), with a normal condition and aspect at the entheses. Bicipital tuberosities were micro-CT scanned (15–17µm) and several portions were analysed in order to reconstruct in 3D the canals of the cortical bone.
Differences were observed in their osseous microarchitectural organisation. In particular, canals were preferentially oriented in "mechanical" EC, while an irregular widening and a higher density characterised "metabolic" EC.
Until further analyses will be performed, the results of this study point towards a possible distinction between different aetiologies of EC, which might represent a valuable contribution to the research on lifestyles and activities in past populations.

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 are mainly encountered at the metaphyseal or diaphyseal areas while fibrocartilaginous entheses include the insertions at the epiphyses and processes of long bones as well as the short bones of hands and feet and several ligaments in the spine.[1]

Entheseal changes (EC) are pathological or non-pathological modifications at the insertion sites.[2] They result in bone alterations visually observable on dry bone. It can take the form of mineralised tissue formation (e.g. irregular surface) and bone formation (raised margin, enthesophyte etc.) as well as surface discontinuity such as fine and macro-porosity, cortical defect, erosive areas, cavitations etc.[3]

EC can be related to age and sex or they can also result from various causes like metabolic or inflammatory disorders, macro-traumas, as well as mechanical stress.[4] Indeed, one of the fundamental roles of an enthesis is stress dissipation, distributing load forces across the bone.[5] Therefore, EC have been considered for decades as occupational stress markers, with the perspective of reconstructing activities and lifestyles of ancient populations.[6] Nevertheless, before the interpretation of EC in terms of possible activities, one might wonder how we can distinguish "mechanical" EC from changes related to other causes. From a direct observation of the changes on the bone, suggesting any specific cause for the observed EC seems to be problematic, especially if there is a lack of information regarding the context and/or the rest of the skeleton.[7]

Entheses and their changes have been extensively studied, with clinical, radiological, histological and osteological methods at the macroscopic and microscopic scales.[8] Recently, the 3D approach has begun to be used as a tool for studying EC, but these researches focus mostly on entheseal surfaces.[9] We chose here the complementary use of micro-tomodensitometry investigation and 3D imaging, which have been little applied to occupational markers so far.[10] In 2015, we already conducted a preliminary exploration of the radial tuberosity of two Neolithic individuals from Mali, in order to perform a first test of the methodology and primary observations of the entheseal microarchitecture.[11] The promising results encouraged us to continue on this path for further studies.

Our aim here is to explore the microarchitecture of the radial tuberosity, in order to identify possible features distinguishing EC on the base of their supposed aetiology. For this exploratory research, we chose to compare EC presumably related to the repeated movement involved in archery, with EC probably caused by a metabolic disorder. Both cases were also compared to the normal aspect of the insertion site. The potential distinction between "mechanical" EC and changes related to other aetiologies would allow us to gain insights into the research on lifestyles and activities in ancient populations.

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1 Benjamin and Ralphs 1998; Benjamin and McGonagle 2001.

2 La Cava 1959; Niepel – Sitʻaj 1979; Lagier 1991; Benjamin et al. 2002.

3 Hawkey – Merbs 1995; Robb 1998; Mariotti et al. 2004; Villotte 2009; Villotte et al. 2016.

4 Dutour 1992; Claudepierre – Voisin 2005; Slobodin et al. 2007; Villotte – Kacki 2009; Jurmain – Villotte 2010; Paja et al. 2010; Milella et al. 2012; Alves Cardoso – Henderson 2013; Henderson – Alves Cardoso 2013; Niinimäki – Baiges Sotos 2013; Villotte – Knüsel 2013; Santana Cabrera et al. 2015; Djukic 2016; Michopoulou et al. 2016.

5 Benjamin and McGonagle 2001.

6 Dutour 1986; Hawkey – Merbs 1995; Pálfi 1997; Peterson 1998; Molnar 2006; Villotte et al. 2010b; Baker et al. 2012; Henderson et al. 2016a.

7 Salmi – Niinimäki 2016.

8 Cooper – Misol 1970; Resnick – Niwayama 1983; Olivieri et al. 1998; Benjamin et al. 2002; Claudepierre – Voisin 2005; Maffulli et al. 2005; Villotte 2009; Junno et al. 2011; Schlecht 2012; Henderson 2013a; Henderson et al. 2016b; Miszkiewicz – Mahoney 2016.

9 Pany et al. 2009; Henderson 2013b; Noldner – Edgar 2013; Nolte – Wilczak 2013; Karakostis – Lorenzo 2016.

10 Djukic et al. 2015; Djukic 2016; Mulder et al. 2016.

11 Berthon et al. 2015a.

Materials

We focused here on *tuberositas radii*, or the bicipital tuberosity of the radius, which is the insertion site of *biceps brachii*, and one of the fibrocartilaginous entheses.[12] These are the most documented group of entheses in the attempt to reconstruct past activities.[13] *Biceps brachii* is one of the flexor and supinator muscles of the elbow, and changes at *tuberositas radii* were previously interpreted to be linked with occupation.[14] Agricultural and building activities, especially carrying heavy loads, have proved to be a potential cause for EC at this enthesis.[15] Thomas[16] investigated the frequency of entheseal changes in a Neolithic population from the Cerny culture (Paris Basin, France). Among 36 identified adult males, 13 were buried in association with arrowheads. Her results reveal a higher frequency of EC at several insertion sites, with a significant difference for the radial tuberosity in particular, among the group of individuals buried with arrowheads. This is one of the studies suggesting archery as an activity prone to lead to EC at this specific insertion site.

We relied on three pairs of radii, belonging to three male adults (Fig. 1).

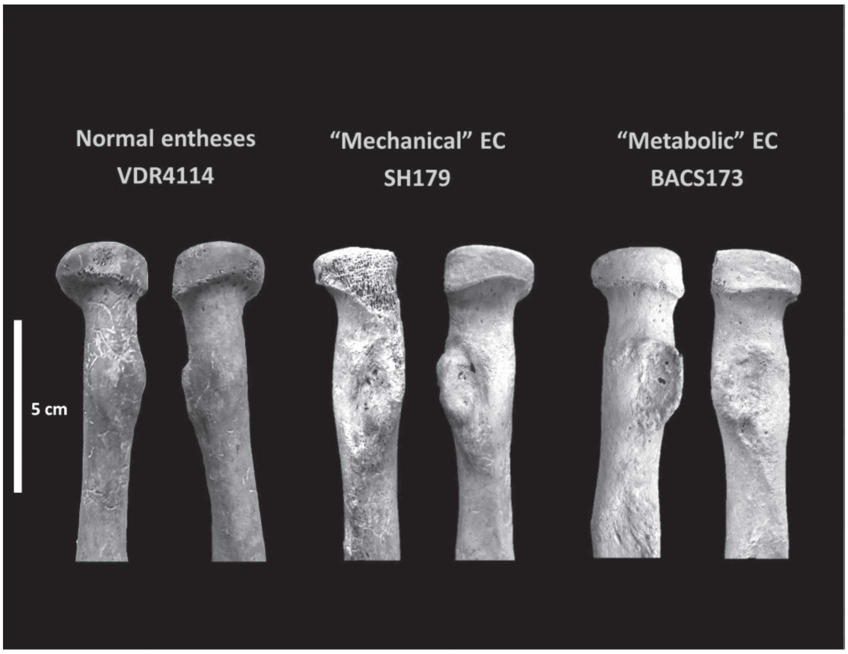

Figure 1.

1) The first individual is presumed to represent the normal aspect of the enthesis. He comes from the Merovingian-Carolingian (VII–Xth Centuries AD) cemetery of Val-de-Reuil "Le Chemin aux Errants", in Normandie, France. The excavation was led by the French National Institute for Preventive Archaeology (INRAP), under the supervision of Yves-Marie Adrian, in 2012. A total of 230 burials were excavated and studied.[17] No evidence for warfare context had been uncovered. The examination of the skeletal remains of the selected individual (VDR4114), a 20–50 years old male,

[12] Benjamin et al. 1986.

[13] Havelková – Villotte 2007; Villotte 2009; Villotte et al. 2010a; Henderson et al. 2013; Villotte – Knüsel 2013; Thomas 2014; Weiss 2015; Henderson et al. 2016c.

[14] Dutour 1986; Hawkey and Merbs 1995; Pálfi 1997; Robb 1998; Molnar 2006; Weiss 2007; Baker et al. 2012; Thomas 2014; Tihanyi et al. 2015.

[15] Commandré 1977; Galera – Garralda 1993; Al-Oumaoui et al. 2004; Havelková et al. 2011; Rojas-Sepúlveda – Dutour 2014.

[16] Thomas 2014.

[17] Beurion 2009; Berthon et al. 2015b.

did not suggest any particular pathological condition or stress prone to lead to bias in the entheseal changes analysis.

Both radial tuberosities showed smooth-rounded contour and smooth and regular surface, despite slight taphonomic alterations likely due to roots in the soil.

2) The second individual is presumed to show activity-related EC at bicipital tuberosities. This mature male comes from the Hungarian Conquest period (X^th Century AD) cemetery of Sárrétudvari Hízóföld. The Hungarian Conquerors of the X^th Century were, according to historical and archaeological data, a population of mounted archers. 263 individuals from that period were excavated under the supervision of Ibolya M. Nepper, between 1983 and 1985, and 58 graves contained weapons, mostly related to archery.[18] This is the case for the selected individual (SH179), with arrowheads and bow elements discovered in association with the skeleton.

The two radiuses of this "presumed archer" exhibited raised margins, bone formation, macro-porosity and fine porosity as well as erosive areas at bicipital tuberosities.

3) The third and last individual has been selected to represent EC probably related to a metabolic condition. This mature male comes from the Hungarian Late Middle Ages – Early Modern time's cemetery (XVI–XVII^th Centuries AD) of Bácsalmás-Homokbánya. This cemetery was excavated in several phases between 1993 and 2003, by Erika Wicker, Zoltán Polgár and László Pintér, and 481 skeletons were unearthed. The archaeological and historical data suggest the presence of a population of farmers, with no evidence for warfare context.[19]

The selected individual (BACS173) was affected by diffuse idiopathic skeletal hyperostosis (DISH) or Forestier's disease. This metabolic disorder is particularly characterised by the calcification and ossification of soft tissues, including ligaments and entheses.[20] The main diagnostic criteria that were observed on the skeleton for this metabolic disorder are: ossification of the right side anterior longitudinal ligament from T2 to L5 (complete and non-complete fusion), with a "candle wax" appearance; normal intervertebral disc spaces; entheseal changes at radii, claviculae, patellae, calcanei or ilii; ossification of rib cartilage and sternocostal ligaments.[21]

The bicipital tuberosities, in particular, were characterised by raised and irregular margins, bone formation, irregular surface and macro-porosity.

Methods

All 6 radii were micro-CT scanned in order to investigate bone microarchitecture of the entheses. Micro-tomodensitometry provides, in a non-destructive way, an insight into the biomechanical properties of bone and the characteristics of bone remodelling through a three-dimensional approach.[22] We applied the micro-computed tomography (micro-CT) acquisitions processing chain[23] 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 Maximum Height) 3D algorithm, allowing the software to automatically identify the optimal limits between each material such as bone and air.[24] The radii were CT scanned at PLACAMAT (UMS 3626, CNRS/University of Bordeaux), Pessac, France, on a GE® Phoenix v|tome|x s, with an isotropic resolution between 15.7 and 17.8 µm. We

18 Nepper 2002; Tihanyi et al. 2015.

19 Lovász et al. 2013.

20 Resnick – Niwayama 1976; Waldron 2009; Holgate – Steyn 2016.

21 Paja et al. 2010; Paja 2012.

22 Lespesailles et al. 2006; Coqueugniot et al. 2010; Colombo 2014; Rittemard et al. 2014; Khoury et al. 2015.

23 Coqueugniot et al. 2011.

24 Spoor et al. 1993; Dutailly et al. 2009.

focused the acquisitions on the enthesis area. The micro-CTs were 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 to globally visualise the entheseal surface for selecting regions of interest (ROIs), on which observations were then performed. In each radius, three portions localised at different height levels (25, 50 and 75%) of the enthesis were selected (Fig. 2). The total height was visually estimated regarding the superior and inferior portions of the margin and considered in terms of number of horizontal slices between these limits. Each bounding box created in this way was 4 mm high, with the medium slice exactly located at the level of interest. The length and width of the boxes depended on the morphology of the bone itself. In general, we selected the ROIs 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 entheseal area.

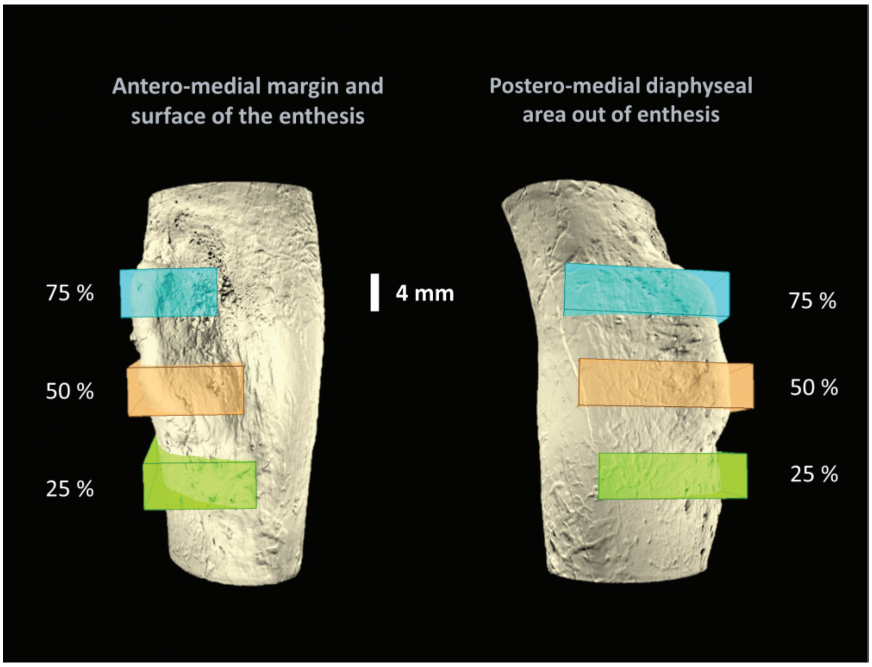

Figure 2.

We then operated segmentation according to the grey level values of each component. It consists of the definition of subsets or materials, in order to make the software able to distinguish bone from empty canals and medullary cavities, external vacuum and sedimentary residues such as sand. Subsequently, a binary image was obtained using 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 HMH algorithm, binary slices were superposed to reconstruct the canal system of the cortical bone, to observe its three-dimensional organisation. This methodology, using micro-CT and 3D reconstructions with TIVMI® software program, has already been performed in a research focusing on trabecular bone microarchitecture during growth, with good repeatability.[25]

[25] Colombo 2014.

Results

From the first observations of the final 3D reconstructions, it appeared that the most relevant level of interest for comparisons between the 3 groups was 50% of the enthesis height. At this medium region of the enthesis indeed, the organisation of the canals of the cortical bone seemed to be less influenced by the morphology of the enthesis itself. At the upper and lower areas, a specific orientation of canals, for example, might be problematic to interpret, because they are transitional locations between a normal flat diaphysis and the most elevated part of the insertion site. Considering this, and in order to make the comparison easier, we decided to present here only the 6 reconstructions performed at this medium level (Fig. 3). The main observations in each case are summarised in Table 1.

The 3D reconstruction of the canal system of the cortical bone, as well as the medullary cavities, concerning a presumed normal enthesis had already been described with more details on a previous case.[26] The "normal" enthesis of this work exhibited a similar type of organisation regarding the canals of the cortical bone. On the medial-posterior face of the shaft, outside the enthesis, we observed a normal Haversian organisation, with thin and longitudinal Havers' canals and a few transversal Volkmann's canals. On the antero-medial margin of the enthesis, the canals, which were thicker, revealed a reticulated organisation with roughly oblique interconnections.

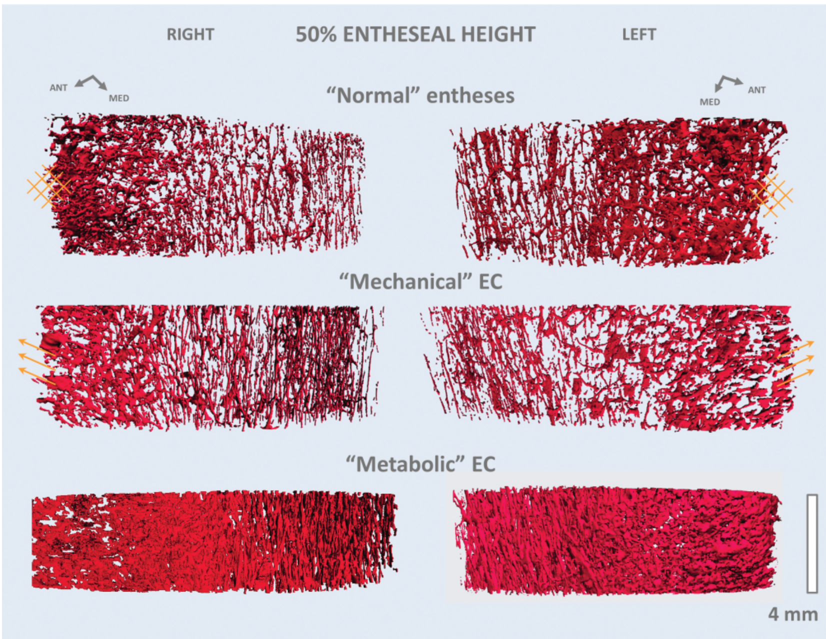

Figure 3.

In the case of the presumed activity-related EC, observed on a probable archer, the medial-posterior face of the diaphysis revealed the same longitudinal and thin organisation of canals. At the antero-medial margin, however, they appeared to be globally oriented toward the same anterior and proximal direction.

The third case, with a DISH condition, showed considerable differences from the two others. Even if the longitudinal organisation was preserved on the diaphysis, the canals were much larger. On the antero-medial face, the reticulation previously observed was not visible anymore. Instead, the organisation appeared to be very irregular, with wide canals present in a higher density.

[26] Berthon et al. 2015a.

	Medial-posterior diaphysis	Antero-medial entheseal margin
"Normal" entheses	Longitudinal; thin	Reticulated; rough
"Mechanical" entheses	Longitudinal; thin	Preferentially oriented
"Metabolic" entheses	Longitudinal; large	Loss of reticulation; irregular; wide; dense

Table 1. Summary of the cortical bone canal organization differences observed between the three groups of entheses

Discussion

Focusing on the organisation of the canal network of the cortical bone, this preliminary exploration of the microarchitecture of an enthesis already allows the identification of variations between the normal condition and EC seemingly related to different causes. We could observe a normal pattern, already identified in a previous work, with a Haversian organisation out of the enthesis and a variation at the entheseal margin. We can assume that the reticulation observed in this case may correspond to the structural adaptation of osteons to normal mechanical constraints, in accordance with Wolff's Law.[27] Concerning the probable archer individual, there is only a slight variation from the normal organisation, involving a bone remodelling with a preferential orientation of the canals. Could we consider this as the reflection of the adaptation to mechanical constraints involved in standardised gestures of specific activities like archery? In this exploratory work, and until further studies will be performed, we can at least support this hypothesis. The last case, with a metabolic disorder, is characterised by the loss of the osteonic organisation inside the entheseal area. This suggests that calcification at entheses resulting from DISH condition might be related to a primary ossification process.

Finally, our preliminary results confirm that the use of micro-CT and 3D imaging can surely enhance our understanding of entheseal changes and their formation. Is it possible to distinguish mechanical and metabolic-related EC? While it is premature to give a definitive answer to this question, we put forward the fact that the observations performed here are promising.

The next steps of this investigation will include larger samples, in order to multiply the observations. The sampling will take into account the numerous biases inherent in studies aiming to reconstruct activities in ancient populations.[28] A better comparative work requires more objective criteriae. We will 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. We obtain in this way a simplified modelling of the cortical microstructure allowing determining qualitative and quantitative parameters.[29] These parameters may be used then to quantify the microarchitecture of normal and changed conditions and to test the interindividual variability. Once the methodology will be well tested and validated, other susceptible aetiologies for EC, such as inflammatory diseases, could be investigated, and various entheses could be analysed. For instance, Djukic in 2016 has been interested in the possible identification of horse riding practice in medieval series, confronting macromorphological observations and micro CT analyses of different EC of the upper and lower limbs.

Besides the methodological aspect of this study, we wish also to build upon the knowledge on the population of Hungarian Conquerors of the Xth Century, in particular about their lifestyles and activities. Questions such as bilateral asymmetry or sex differentiation, analysed under this

[27] Frost 1994; Djukic 2016.

[28] Dutour 1992, 2000; Villotte 2009; Meyer et al. 2011; Jurmain et al. 2012; Milella et al. 2012; Alves Cardoso – Henderson 2013; Perréard Lopreno et al. 2013; Thomas 2014.

[29] Colombo 2014.

framework, might open up some interesting horizons for the research field on activities reconstruction but also regarding various cultural aspects among these tribes.

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List of journal abbreviations

Acta Biol Szeged:	Acta Biologica Szegediensis
Am J Phys Anthropol:	American Journal of Physical Anthropology
Angle Orthod:	The Angle Orthodontist
Anthropol Sci:	Anthropological Science
Bailliere's Clin Rheum:	Bailliere's Clinical Rheumatology
BMSAP:	Bulletins et Mémoires de la Société d'Anthropologie de Paris
Chungará (Arica):	Chungará: Revista de Antropología Chilena
Clin Anat:	Clinical Anatomy
Clin Rheum Dis:	Clinics in Rheumatic Diseases
Coll Anthropol:	Collegium Anthropologicum
Comp Biochem Physiol:	Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology
A Mol Integr Physiol	Connective Tissue Research
Connect Tissue Res:	
HOMO:	HOMO Journal of Comparative Human Biology
Int J Anthropol:	International Journal of Anthropology
Int J Osteoarchaeol:	International Journal of Osteoarchaeology
Int J Paleopathol:	International Journal of Paleopathology
J Anat:	Journal of Anatomy
J Bone Joint Surg Am:	Journal of Bone & Joint Surgery
J Hum Evol:	Journal of Human Evolution
Presse Med:	La Presse Médicale
Rev Rhum:	Revue du Rhumatisme
Semin Arthritis Rheum:	Seminars in Arthritis and Rheumatism

Figures