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► To cite this version:

Sébastien Villotte, Frédéric Santos. The effect of age on enthesal changes: A study of modifications at appendicular attachment sites in a large sample of identified human skeletons. *International Journal of Osteoarchaeology*, 2022, 10.1002/oa.3197 . hal-04021650

HAL Id: hal-04021650

<https://hal.science/hal-04021650>

Submitted on 9 Mar 2023

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Title The effect of age on enthesal changes: a study of modifications at appendicular attachment sites in a large sample of identified human skeletons

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Running title: The effect of age on enthesal changes

Key words: Age-at-death; fibrous enthesis; fibrocartilaginous enthesis; musculoskeletal stress markers; enthesal changes; Spearman's rank correlation coefficient; Fisher exact test; mode; relative stage frequency; stacked bar plot; mosaic plot; logistic regression model; log-linear model; adjusted standardized residual; generalized odds ratio.

Abstract:

Entheseal changes (EC) are frequent in older individuals, and age-at-death is the main variable explaining the distribution of EC stages in adult skeletal samples. While this is already well known, the underlying process has not been studied in biological anthropology to date. The aim of the article is to describe the relationship between age and stages of EC in detail for 30 appendicular entheses in a large sample of adult skeletons (N=721) with known age-at-death and sex, using several statistical tools (such as Spearman's rank correlation coefficients, logistic regression models, adjusted standardized residuals, and generalized odds ratios). The analyses indicate a moderate to strong relationship between age-at-death and EC for all the appendicular attachment sites, but also striking differences between fibrous and fibrocartilaginous enthesal attachment sites. These differences are discussed in light of the available biomedical data.

1. Introduction

The study of skeletal changes at entheses (i.e., tendon and ligament attachment sites) has a long history. Early on, these changes were directly linked to muscular recruitment, often in a rather simplistic cause and effect relationship (see Jurmain et al., 2012). Since then, many scholars have expressed concerns about the validity of this approach, highlighting several factors that influence the appearance of such changes, the main one being age (Dutour, 1992; Jurmain et al., 2012; Villotte & Knüsel, 2013). In identified skeletal collections, it has been shown repeatedly that age-at-death is the main variable explaining the distribution of enthesal changes (EC) in adults (Cardoso & Henderson, 2010; Cunha & Umbelino, 1995; Henderson et al., 2017; Listi, 2016; Mariotti et al., 2004, 2007; Milella et al., 2012; Niinimäki, 2011; Shaibani et al., 1993; Takigawa, 2014; Villotte et al., 2010a). When Pearson correlation coefficients are provided, a substantial part of variation (ca. 20%, but up to 48%) is explained by age alone (Table 1). While the influence of age is now integrated in most studies of EC in bioarchaeology, the underlying process is not well understood and, at present, does not appear to have been properly studied in biological anthropology.

<INSERT TABLE 1 HERE>

For some bioarchaeologists, skeletal changes gradually appear throughout the life course and become more and more extreme due to the accumulation of stress produced by physical activity (e.g., Alioto, 2020; Robb, 1998). Alioto (2020, p. 184) considers, for instance, that “older individuals will have built up stronger attachment sites over time than younger individuals.” It seems that for these scholars, most EC are “physiological”, and the mechanism behind the appearance of enthesal changes can be ascribed to “muscle use”. According to other scholars who consider skeletal changes at entheses as pathological (and call them enthesopathies), at least two phases can be identified during adulthood: an early phase where overuse can induce microtrauma, and a second phase in adulthood, starting during the 6th decade of life, marked by a generalized degeneration of the soft and hard tissues (e.g., Dutour, 1986; Villotte et al., 2010b). This apparent discrepancy is, in our opinion, likely related to the

nature of the entheses analyzed in these studies: both fibrocartilaginous and fibrous entheses in the first case and only fibrocartilaginous attachment sites in the second.

The relevance of the distinction of fibrous and fibrocartilaginous entheses in biological anthropology has been previously discussed in detail (e.g., Jurmain et al., 2012; Villotte & Knüsel, 2013). Briefly, in fibrous muscular attachment sites, which occur typically on the diaphysis of long bones, collagen fibers from tendons or ligaments attach to bone directly or via a mediating layer of periosteum (Benjamin et al., 2002). The surface of such entheses is usually irregular and poorly delimited on adult skeletal remains. Lesions at fibrous attachment sites are rarely mentioned in the medical literature (for a review, see Henderson, 2013; Villotte, 2009), justifying avoidance of the term “enthesopathy” for the EC frequently seen at these sites. Fibrocartilaginous entheses are mostly found at long bone extremities and are characterized by two mediating layers, uncalcified and calcified fibrocartilage, between tendon/ligament fibers and bone. These two fibrocartilaginous layers are avascular and separated from each other by a regular calcification front, which represents the surface observed on dry bones (Benjamin et al., 2002). The role of age, mechanical stresses, and systemic conditions (such as spondyloarthropathies) in the occurrence of enthesopathies at fibrocartilaginous sites is relatively well documented in the biomedical literature (for a review, see Jurmain et al., 2012; Villotte, 2009; Villotte & Knüsel, 2013).

At first glance, the relationship between age and EC does not appear to dramatically differ between fibrous and fibrocartilaginous entheses in identified skeletal collections (Listi, 2016; Milella et al., 2020; Takigawa, 2014; Villotte, 2009). However, it seems that this subject has only been studied superficially to date. The aim of this study is thus to describe in detail the relationship between age-at-death and changes at fibrocartilaginous and fibrous enthesal attachment sites in a large sample of skeletons of known age-at-death and sex. Previous analyses (Villotte, 2009; Villotte et al. 2010a) have shown that a strong and significant positive correlation exists between age-at-death and EC stages in this skeletal sample. In order to better understand the underlying processes governing the appearance of such changes, the hypotheses we aim to test here are the following:

- 1) Considering the anatomical differences between fibrous and fibrocartilaginous entheses, the relationship between age-at-death and EC stages should not be identical in the two groups;
- 2) In our sample, the distribution of EC at fibrocartilaginous attachment sites should be in accordance with medical data (e.g. Bakirci et al., 2020; Bard, 2003; Durigon & Paolaggi, 1991; Rodineau, 1991), with major skeletal changes mainly seen in individuals who died after the 5th decade of life;
- 3) In our sample, the distribution of EC at fibrous attachment sites may reflect a more gradual increase in skeletal changes throughout the life-course.

2. Material and methods

2.1. Identified skeletal collections

The sample includes skeletons of Europeans who died between the 18th and 20th centuries, from four collections: the Christ Church Spitalfields Collection at the British Museum of Natural History, London (UK) (Molleson & Cox, 1993); the identified skeletal collection of the Anthropological Museum of the University of Coimbra, Portugal (Rocha, 1995; Santos, 2000); and the Sassari and Bologna collections of the Museum of Anthropology, University of Bologna, Italy (Facchini et al., 2006). Personal information (age-at-death, sex, cause-of-death, occupation...) is available for most of these individuals, but only the age-at-death and sex were considered in the analyses of the present study. Only skeletally mature individuals (arbitrarily fixed at an age-at-death of 20 years or over) were included in this study. Poorly preserved skeletons, as well as pathological specimens, especially cases of diffuse idiopathic skeletal hyperostosis and seronegative spondyloarthropathy (for a detailed presentation of the criteria used, see Villotte, 2009; Villotte et al., 2010a), were excluded. Fractured limb bones were not scored. Ultimately, 721 subjects were selected for this study (Table 2). The data set is available at <https://zenodo.org/record/6805623>.

<INSERT TABLE 2 HERE>

2.2. Recording method

Thirty (12 fibrous and 18 fibrocartilaginous) appendicular entheses were studied using an ordinal scale with three scoring stages (Villotte, 2006). Here, we briefly present these stages for both groups of entheses (for definitions and illustrations of the terms used, see Villotte, 2006; Villotte et al., 2016).

The 12 fibrous entheses are presented in the Table 3. Changes were recorded for this group (group 4 in Villotte 2006) as follows:

Stage A: overall smooth surface;

Stage B: diffuse cortical irregularity for the major part of the attachment area, but without osseous longitudinal protrusions;

Stage C: longitudinal protrusion(s), such as crests, associated with diffuse cortical irregularity.

In few cases, and only for the attachment of the *M. pectoralis major*, cortical surface discontinuities were also recorded (see Villotte 2006).

The 18 fibrocartilaginous entheses under study are presented in the Table 4. Changes were recorded for this group (group 1 in Villotte 2006) as follows:

Stage A: the site of attachment is smooth, well circumscribed and devoid of vascular foramina;

Stage B: Slight modifications affecting the margin (irregularities or enthesophyte(s)), and/or the inner part (irregularities, foramina, cystic changes, calcified deposits, bony production, or osseous defects) of the enthesis;

Stage C: Major modifications affecting both the margin and the inner part of the enthesis.

2.3. Statistical analyses

In previous studies (Villotte, 2009; Villotte et al., 2010a), this data set was (completely or partially) analyzed using generalized estimating equations. While such an approach seems appropriate in order to describe the overall effect of contributing factors, such as age-at-death and occupation, at the individual level, we preferred a different approach in this study. Using several statistical methods, we analyzed the relationship between stages in EC and age-at-death for each of the 30 entheses, but

illustrated in detail only the results for one fibrous (the left deltoid tuberosity) and one fibrocartilaginous (the left lateral epicondyle of the humerus) attachment sites. These entheses were chosen as they are: 1) frequently studied in bioarchaeology, and 2) “typical” of each type of attachment site.

In this study focusing on the effect of age on enthesal modification, we used the information about age-at-death as both a continuous and a categorical (age groups) variable, depending on the analysis employed. Five age groups (Table 2) were arbitrarily defined in order to obtain similar numbers of individuals in each group. The mode and relative stage frequency were used to describe the distribution of the EC data for each enthesis, and its relationship with age-at-death was assessed using Spearman’s rank correlation coefficients and Fisher exact tests. Distributions of changes according to age groups were illustrated using stacked bar plots, and mosaic plots (Friendly, 1994) were created to visualize the effect of sex on these distributions.

For each attachment site, an ordinal logistic regression model (Agresti, 2002) was defined, with the trichotomic enthesal stage as the ordered response variable, and age group, sex and their interaction as explanatory variables. For each ordinal logistic regression model, the main effects of age and sex, along with the effect of their interaction terms, were tested in an analysis of deviance table using type II tests. Alternatively, log-linear models (Christensen, 1997) were built to assess the significance of the interaction between sex, age group and enthesal changes for each locus; theoretical details and results are provided in Supporting Information A, section 4.4.2.

We also used several approaches described in Sharpe (2015) in order to determine the source of statistically significant differences between age groups. Specifically, we computed “adjusted standardized residuals”, as defined in Sharpe (2015), in order to identify specific cells making the greatest contribution to chi-square test results. When there are many cells (as in our analyses), an absolute value for an “adjusted standardized residual” exceeding about 3 is a good indicator of lack of fit of H_0 for that specific cell. We thus used that value as a guide to which cells were of interest.

In order to compare the distributions of enthesal changes (considered as ordinal variables) across two given age groups, Fisher exact tests were performed and generalized odds ratios (Agresti, 1980) were computed. A generalized odds ratio expresses the probability that enthesal changes are stochastically greater in one given age group; i.e., it is equal to the ratio $P(X > Y) / P(Y > X)$, where X is the random variable for enthesal changes in the first group and Y the random variable for enthesal changes in the second group.

All statistical analyses were performed using R 4.2.1 (R Core Team, 2022). For all tests, the significance threshold was set to $\alpha = 0.05$. A PDF export of a notebook created with Org mode 9.5.5 (Schulte et al., 2012) contains all corresponding R codes, and can be found online as Supporting Information A.

<INSERT TABLES 3 AND 4 HERE>

3. Results

3.1. Frequencies, and their relationship with age-at-death in the entire sample

For all fibrocartilaginous sites, changes are relatively rare in the whole sample: the mode corresponds to stage A. In comparison, changes at fibrous entheses appear far more common, with stage B as the mode for all fibrous sites, except for the tibial origin of the *M. soleus* (Table 3).

Figure 1 presents the bar plots for the left lateral epicondyle and the left deltoid tuberosity of the humerus as examples of trends seen for fibrocartilaginous and fibrous entheses, respectively (bar plots for the other entheses are available in the online as Supporting Information B). For both types of entheses, changes tend to be more common in older age groups (Fig. 1). This is illustrated by a positive, significant, and moderately strong correlation between age (as a continuous variable) and ordinal stages, which is seen for all (fibrous and fibrocartilaginous) entheses under study (Tables 3 and 4).

<INSERT FIG 1 HERE>

While the correlation coefficients between age-at-death and stages of enthesal changes are similar for fibrocartilaginous and fibrous sites, the distribution of enthesal changes in age groups differ completely, as observed graphically between the two groups of entheses (Fig. 1 and Supporting Information B). For each fibrocartilaginous site, the percentage of individuals exhibiting enthesal changes grows roughly exponentially across age classes (most of the changes appearing in the oldest age classes); whereas this percentage grows roughly logarithmically across age classes for fibrous entheses, reaching a plateau after a substantial increase when compared with the youngest age classes.

Chi-square tests for each enthesis indicate that stages and age groups are related ($P < 0.001$, results not shown). For the deltoid tuberosity, when the distribution of stages A, B and C by age group is examined in detail, and based on the analysis of the adjusted standardized residuals, there are more entheses scored as stage A in the age group [20-29] and fewer entheses scored as stage A in the age groups after 40 years than would be expected by chance (see Section 5.1 in Supporting Information A). Regarding stages B and C, they are more frequent than expected in the [60-99] age group and less frequent than expected in the [20-29] age group. For the left lateral epicondyle, there are more stage A scores in the age group [20-29] and fewer entheses scored as stage A in the age groups after 50 years than would be expected by chance (see Section 5.1 in Supporting Information A). There are fewer entheses scored as stage B before 50 years than expected, and more entheses scored as stages B and C than would be expected by chance in the age group [60-99].

3.2. Ordinal logistic regressions for each enthesis

Results of models testing the main effects of the two covariates (age and sex), along with their interaction terms, for each enthesis, are summarized in Table 5. At a significance level of 0.05, the effect of age-at-death (as a continuous variable) is highly significant in all models, the effect of sex is significant for 10 (out of 12) fibrous entheses, and 13 (out of 18) fibrocartilaginous attachment sites. The effect of the interaction of these two covariates is significant in relatively few (4 out of 30) models.

Similar results were obtained when age groups (as a categorical variable) were used instead of age classes as the response variable, although a few models associated with the entheses with the highest proportions of missing values did not converge correctly in this case (results not shown). Overall, changes are more frequent in older individuals for all entheses and tend to be more frequent in males than in females for most of them, regardless of the nature of the enthesis (Table 5).

<INSERT TABLE 5 HERE>

3.3. Models and *post-hoc* analyses for the left deltoid tuberosity of the humerus

Figure 2 presents a mosaic plot describing the distribution of stages by age groups and sex for the left deltoid tuberosity of the humerus.

3.3.1. Main effects of sex

In an ordinal logistic regression predicting enthesal changes for the left deltoid tuberosity, the main effects of age group and sex are found to be significant ($P < 0.001$ in both cases), whereas the interaction was not ($P = 0.852$). These results are identical to those obtained when raw age (in years) is used instead of age groups (full results are given in Supporting Information A, Section 4.4.1).

When adjusted for age, males tend to display more changes than females: for each age group, changes are more frequent in males (Figure 2). Moreover, major changes (stage C) seem to appear earlier in life in the male sample than in the female sample (Figure 3).

<INSERT FIGURES 2 AND 3 HERE>

3.3.2. Main effects of age

Table 6 presents the results of Fisher exact tests and generalized odds ratios for each comparison of paired age groups for the male sample. The highest odds ratio is seen for the comparison between the [20-29] and [30-39] age groups (Gen OR = 2.23; 95% CI = [1.62, 3.05]), and then the values decrease regularly down to ca. 1 in subsequent pairwise comparisons of consecutive age groups. These results can be interpreted as a very rapid increase in frequencies of changes (most at stage B) during early adulthood (i.e., before 40 years old) followed by a plateau (Figure 2). As for males, the main differences in pairs of consecutive age-groups are seen in early adulthood in the female sample, although the increase in enthesal change scores appear more regular (Figure 2), with no significant differences between [30-39] and [40-49], [40-49] and [50-59], and [49-59] and [60-99] age groups (Table 7).

<INSERT TABLES 6 AND 7 HERE>

3.4. Models and *post-hoc* analyses for the left lateral epicondyle of the humerus

Figure 4 is a mosaic plot describing the distribution of EC stages by age and sex for the left lateral epicondyle.

3.4.1. Main effects of sex

In an ordinal logistic regression model predicting enthesal changes for the left lateral epicondyle, the main effects and age group and sex are significant ($P < 0.001$), whereas the interaction is not ($P = 0.748$). Once again, the overall conclusions remain unchanged when using raw age (in years) instead of age groups. When adjusted for age, females tend to display greater changes than males. This is especially clear after 40 years of age (Figure 4). However, major changes (stage C) seem to appear slightly earlier in life for males than for females (Figure 3).

<INSERT FIGURE 4 HERE>

3.4.2. Main effects of age

Table 8 presents the results of Fisher exact tests and generalized odds ratios for each comparison of paired age groups for the male sample. These results indicate very similar (and relatively low, see Figure 4) frequencies of EC in age groups before 50 years of age, and a significant, dramatic and regular increase of changes after that age. Very similar results were obtained for the female sample (Table 9), although the increase in changes with age appears more regular than for males, both graphically and based on the values of generalized odd ratios. In both sexes, major changes appear after 50 years of age (Figures 3 and 4).

<INSERT TABLES 8 AND 9 HERE>

4. Discussion and conclusions

Several variables have been identified as possibly or probably influential factors that affect the frequencies of EC in archaeological samples, including physical activities, mobility patterns and body mass (e.g. Carballo-Pérez et al., 2021; Godde et al., 2018; Laffranchi et al., 2020; Refai, 2019; Sparacello et al., 2020), but age-at-death remains the main factor explaining EC distribution in human skeletal samples. In our study, stages clearly correlate with age for both fibrocartilaginous and fibrous entheses. This result is in agreement with previous analyses of EC in identified collections (Table 1). As noted previously by many other scholars (Cardoso & Henderson, 2010; Cunha & Umbelino, 1995; Henderson et al., 2017; Listi, 2016; Mariotti et al., 2004, 2007; Milella et al., 2012; Niinimäki, 2011; Shaibani et al., 1993; Takigawa, 2014; Villotte et al., 2010a), age appears to be an important (if not the most important) factor influencing the appearance of changes at entheses.

Fibrous and fibrocartilaginous attachment sites clearly differ in our analyses in terms of frequencies of EC (much more frequent for fibrous entheses), but also in terms of the relation between EC and age-at-death. Based on the data presented in Tables 2 and 3, and Supporting Information B, it appears that fibrous and fibrocartilaginous entheses form two very distinct groups. Before discussing the patterns

of aging identified for these two groups of entheses in detail, some limitations of our study should be highlighted.

In this study, we applied a specific scoring system to each group of entheses (see Villotte 2006). It is thus possible that a portion of the differences seen between fibrous and fibrocartilaginous entheses in our analyses are the consequence of methodological choices. Nevertheless, it seems also likely, taking into account the large number of anatomical and pathological differences between fibrous and fibrocartilaginous attachment sites, that these results are, in some measure, also linked to their very different natures, and thus reflect real biological processes. As a consequence, considering that similar enthesal change “scores” for several attachment sites as indicative of a group of muscles working together (e.g. Alioto, 2020; Hawkey & Merbs, 1995) appears problematical when both types of entheses are studied together: fibrous entheses will group together and tend to display higher ranks than fibrocartilaginous ones (see also Villotte 2009 for other issues regarding the effect of this approach). Considering the obvious differences illustrated here, inferences based on direct comparisons of stages between fibrous and fibrocartilaginous entheses should be avoided in our opinion.

Another limit of our study on the effect of age on EC distribution is the lack of control for factors other than sex. This choice permitted us to carry out many different analyses and to present our results in detail, but also limits the scope of the study. As skeletal age changes are not uniform across populations (e.g. Katz & Suchey, 1989; Schmitt et al., 2002), pooling four collections of different origins and with different demographic profiles may have had an impact on our results. However, previous studies of this sample have shown that the “collection” factor has few effects on the distribution of EC (Villotte 2009, Villotte et al. 2010a), likely due to the fact that European groups seems to be rather homogenous with regard to skeletal changes associated with aging (Schmitt et al., 2002). It thus appears relevant to consider an expanded sample including extra-European collections on the effect of age on EC in subsequent studies. Body mass has been identified as a possible factor influencing frequency distributions of EC (mostly for fibrous entheses, see for instance Godde et al., 2018). Body

mass is not known for the individuals studied here and the reliability of current methods to estimate body mass from the skeleton remains poor (Lacoste Jeanson et al., 2017), preventing the analysis of this factor in these collections. Identified skeletal collections with known individual body masses should be taken into account in further studies in order to look for a possible interaction between age and body mass. Finally, we did not attempt to include occupations, which are known for most of the males studied here (see Villotte 2009, Villotte et al. 2010a), for three reasons. First, whereas the variable "occupation" does explain a part of the distribution of EC in identified samples (e.g. Villotte et al. 2010a; Milella et al., 2012; Alves Cardoso & Henderson, 2013; Milella et al., 2015), its effect is clearly smaller than is that for age. Second, it has been shown that the way occupations are categorized may affect the results (Alves Cardoso & Henderson, 2013), creating potential biases. Finally, in the collections studied here, descriptions of female occupations are scarce and hard to interpret with regard to physical activities (Molleson & Cox, 1993; Cunha & Umbelino, 1995; Villotte et al., 2010a), preventing analyses on the complete dataset. It would certainly be beneficial to include all these factors (and likely others) in further analyses, but the aim of this paper was to clarify first and foremost the relationship between EC and age-at-death, a relationship which has never been studied in detail previously.

For fibrous entheses, the frequency of enthesal changes (stages B and C) increased rapidly during the third and fourth decades of life and then stabilized (around ca. 70% for the attachment of *M. deltoideus*) in later age groups. Most of the changes before 40 years old correspond to stage B (i.e., diffuse cortical irregularity). Sometimes called "robusticity", diffuse cortical irregularity at fibrous entheses has long been considered the product of bone remodeling stimulated by repeated - normal or strenuous- muscle contractions during adulthood. Even if this hypothetical relationship seems reasonable, it is based on almost no medical or experimental data (see Villotte & Knüsel, 2013). In immature animals, tendons and ligaments attach to the diaphysis via the periosteum, and the periosteal layer may or may not disappear (depending on the species and the enthesis) when growth stops (e.g. Gao et al., 1996; Hurov, 1986; Matyas et al., 1990). In mature humans, the mediating layer

of periosteum is absent in most appendicular fibrous entheses and the soft tissue attaches directly to bone (Benjamin et al., 2002), except where muscles attach to a large area by short fibrous tissues (Kenesi & Tallineau, 1991). At what point during life the transition from a periosteal to a bony attachment occurs has not been documented in humans. Based on the fact that adolescent skeletons exhibit specific features at fibrous entheses that are not observed in adults, it has been hypothesized that this physiological transition also occurs when growth stops in humans (Villotte & Knüsel, 2013). This may explain (combined or not with the potential effect of muscular contractions) the increasing frequency of skeletal changes (i.e., “diffuse cortical irregularity”) seen in young/middle-aged adults. The frequency of stage C for fibrous entheses increases regularly during adulthood. As the frequency of enthesal changes, stages B and C, reach a plateau after the fifth or sixth decade, it seems that major changes, as represented by stage C, progressively replace more minor ones (stage B) in older individuals. Stage C, as represented by crests or other bony protrusions at fibrous entheses, appear to be in direct and progressive continuity with diffuse cortical irregularity, and thus the “muscle use” hypothesis may be valid for these attachment sites. In view of the paucity of histological and biomedical data for these sites, this interpretation does not imply a direct cause and effect relationship between physical activity and EC at fibrous entheses. The “muscle use” hypothesis may be useful as a theoretical framework for these changes, but more histological and experimental analyses are required before considering EC at fibrous entheses as reliable activity-related skeletal changes.

For fibrocartilaginous sites, the percentage of individuals exhibiting enthesal changes grows exponentially across age classes, with most changes appearing in the older age classes. The turning point seems to occur in the 5th or 6th decade of life, when significant differences appear in comparison with those of previous age groups. Major changes as represented by stage C also appear around this period of life, and become frequent (i.e., more than would be expected by chance) after 60 years of age. This result is in agreement with biomedical data available for appendicular fibrocartilaginous entheses. In young/mature healthy adults, lesions at the enthesis are uncommon and often mechanically induced when they do occur (Bard, 2003; Rodineau, 1991, see Villotte & Knüsel, 2013 for

a review). These enthesopathies are generally associated with minor skeletal changes. From the sixth decade onwards, a generalized disruption of the soft and hard tissues and the appearance of major skeletal changes is observed and, during this second phase, mechanical factors play only (if any) a secondary role in the appearance of lesions (Bakirci et al., 2020; Bard, 2003; Durigon & Paolaggi, 1991; Rodineau, 1991). It has been hypothesized that this rapid appearance of major skeletal changes may be exacerbated in women (Villotte, 2009; Villotte & Knüsel, 2013) by a drop in circulating estrogen at the menopause and the related changes in the composition of collagen connective tissues (e.g. Falconer et al., 1996). However, the effect of the interaction of age and sex was significant for only one fibrocartilaginous attachment site (out of 18) in our analyses (table 5). Further studies seem necessary in order to better understand the effect of the interaction between hormones and age in the appearance of skeletal EC.

It should be noted that, contrary to the situation in fibrous entheses, the increase in frequency of stage C with age is not achieved to the detriment of the frequencies of stage B (for which the frequency also increases in older age groups) in our study. Based on the empirical data presented here and the medical data available, it seems possible that stage C appeared, for the most part, relatively quickly from a previously healthy (i.e. unmodified) enthesis (i.e., stage A) in individuals over 50 years of age. Then, it appears that the “muscle use” hypothesis should be rejected in the case of fibrocartilaginous entheses. As noted previously (Villotte & Knüsel, 2013), EC seen in older individuals are probably not directly related to physical activity, and certainly not only to micro-trauma at the enthesis. On the contrary, minor changes at fibrocartilaginous entheses in early/middle adult skeletons may be good indicators of microtrauma during life.

To sum up, detailed statistical analyses permitted us to illustrate two different patterns of relationships between EC and age according to the nature of the attachment site. The patterns seem to be related to physiological and pathological processes that should be taken into account when studying EC in archaeological human skeletal samples. Moreover, the two groups of entheses appear very homogeneous with regard to the effect of age on the appearance of EC. Further analyses of each type

of enthesis are required in order to identify what other factors interact with age in order to explain the distribution of EC in skeletal samples.

5. Acknowledgements

S.V. thanks Sarah Schrader and Jared Carballo Pérez for inviting him to the “Adaptive Tools for Resilient Bones” conference. The authors thank the three reviewers for their useful comments and Christopher Knüsel for his editorial assistance with the English expression of this contribution. For proofreading the article. S.V. is grateful to the curators and staff of the Natural History Museum in London, the Department of Anthropology of the University of Coimbra, and the Department of Anthropology of the University of Bologna for providing access to the material and for their helpful comments on the collections. Study of the skeletal collections were partly funded by the European Union (Synthesys, grant number GB-TAF-636), and the French Ministry of Higher Education, Research and Innovation (“Aires Culturelles”). The research was carried out during a project funded by the CNRS (International Emerging Actions, grant number IEA00540). Both authors declare that they have no conflicts of interest.

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