

1 **Exploring the relationship between enthesal changes and physical activity: a**
2 **multivariate study**

3 Marco Milella¹, Francisca Alves Cardoso², Sandra Assis³, Geneviève Perréard Lopreno⁴, and
4 Nivien Speith⁵

5
6 ¹Anthropological Institute & Museum, University of Zurich

7 ²CRIA - Centro em Rede de Investigação em Antropologia, Faculdade de Ciências Sociais e
8 Humanas, Universidade Nova de Lisboa

9 ²CIAS - Research Centre for Anthropology and Health, University of Coimbra

10 ⁴Laboratory of prehistoric archaeology and anthropology, University of Geneva

11 ⁵Faculty of Science and Technology, Bournemouth University

12

13 Number of pages: 20 (including references).

14 Number of tables: 4 (plus 2 tables in Supplementary Material)

15 Number of figures: 3 (plus 4 figures in Supplementary Material)

16 Abbreviated title: A multivariate study of enthesal robusticity

17 Keywords: Enthesal robusticity, Occupations, Multivariate study

18 **Please send proof to:** Dr. Marco Milella, Anthropological Institute & Museum, University of
19 Zurich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

20 E-mail: marco.milella@aim.uzh.ch

21

22 **ABSTRACT**

23 Analyses of enthesal changes (EC) in identified skeletal samples employ a common research
24 strategy based on the comparison between occupations grouped on the basis of shared
25 biomechanical and/or social characteristics. Results from this approach are often ambiguous,
26 with some studies that point to differences in EC between occupational samples and others
27 failing to provide evidence of behavioral effects on EC. Here we investigate patterns of EC
28 among documented occupations by means of a multivariate analysis of robusticity scores in
29 nine postcranial entheses from a large (N=372) contemporary skeletal sample including
30 specimens from one Italian and two Portuguese identified collections. Data on enthesal
31 robusticity, analyzed by pooled sides as well by separated sides and levels of asymmetry, are
32 converted in binary scores and then analyzed through nonlinear principal component analysis
33 and hierarchical cluster analysis. Results of these analyses are then used for the classification
34 of occupations. Differences between occupational classes are tested by MANOVA and
35 pairwise Hotelling's test. Results evidence three classes which separate occupations related to
36 farming, physically demanding but generalized occupation, and physically undemanding
37 occupations, with the more consistent differences between the first and the last classes. Our
38 results are consistent with differences in biomechanical behavior between the occupations
39 included in each class, and point to the physical and social specificity of farming activities.
40 On the other hand, our study exemplifies the usefulness of alternative analytical protocols for
41 the investigation of EC, and the value of research designs devoid of *a priori* assumptions for
42 the test of biocultural hypotheses.

INTRODUCTION

46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70

The last decades have seen a resurgence of studies on enthesal changes (EC) and their reliability as “skeletal markers of activity” (Jurmain 1999; Jurmain et al. 2012 and authors therein). The analysis of identified human skeletal collections (IHSC) played a key role in this regard, allowing to test the effects of variables like age, sex and biomechanical stress on the expression of EC (Alves Cardoso and Henderson 2010; Belcastro et al. 2007; Mariotti et al. 2004; Milella et al. 2012; Niinimäki 2011; Niinimäki and Baiges Sotos 2013; Villotte et al. 2010). While some studies confirmed a correlation between EC and biomechanical stress (Niinimäki 2011; Villotte 2009; Villotte et al. 2010 – but only for fibrocartilaginous entheses), others highlighted the overruling role played by physiological features such as age and sex on EC variance (Alves Cardoso and Henderson 2010, 2013; Henderson et al. 2013; Milella et al. 2012; Perréard Lopreno et al. 2013; Villotte 2009 - but mainly for fibrous entheses). Contrasting results on the reliability of EC as skeletal markers of activity can be linked to methodological differences (e.g. inter-observer differences in scoring methods and/or in investigated sites) as well as to different approaches used for controlling age, sex, and other relevant factors (e.g. body size, body mass).

Most anthropological studies on IHSC classify occupation according to either socio-cultural or biomechanical criteria. A socio-cultural criterion – the division of tasks based on the concept of gender and their male and female categorization – was explicitly chosen by Alves Cardoso (2008) when testing the correlation between EC and degenerative joint changes (DJC) and known occupations. The assumption underlying this research was that the EC and DJC would reflect the sexual division of labor that would mirror gender constructs associated with sex-specific tasks performed by male and female individuals. The biological sex was therefore used as a proxy to gender, mediated by changes in the skeletons which were defined as markers of occupational stress: but gender and sex were always understood as

71 separate categories. The socio-cultural criterion was chosen in order to allow inferences on
72 possible gender differences in habitual activities in Portugal between the 19th and 20th
73 centuries to explore this topic in a framework that allowed controlling variables capable of
74 biasing the research, such as: sex, age at death and occupation.

75 To date, however, the more extensively used criterion is biomechanics, which
76 classifies occupation according to the type and degree of associated biomechanical strain
77 (Milella et al. 2012; Niinimäki and Baiges Sotos 2013; Villotte et al. 2010). Despite a shared
78 theoretical background, biomechanical classifications vary largely among authors (Perréard
79 Lopreno et al. 2013), and therefore prevent comparisons between different studies. In this
80 sense, biomechanical criteria appear often affected by the same subjectivity biasing socio-
81 cultural criteria. Another issue common to most studies on IHSC is the possible bias
82 introduced by the adopted classification for the results of the study. The research design
83 shared by most studies on EC consists in testing the consistency between patterns of EC and
84 assumed social or biomechanical categories, by employing customary traditional parametric
85 or nonparametric univariate and bivariate statistical protocols. However, the reliability of the
86 chosen categories (i.e. their real consistency with the lifestyle and life history of the subject) is
87 generally unverifiable, especially due to the inconsistent documentary information available
88 for most of past occupations. Accordingly, this approach is prone to mask the real patterns
89 underlying the data, leading to problems in the interpretation of the results. An alternative
90 approach consists of classifying the various occupations based on the observed similarities
91 between individuals without *a priori* established categories. Such a strategy would allow a
92 subdivision of the sample that is much more consistent with the observed patterns of changes,
93 therefore avoiding problems represented by both social and biomechanical classifications.
94 From a statistical point of view, the use of eigenvector-based multivariate procedures (e.g.
95 principal component analysis - PCA) seems particularly suited for an approach that aims to

96 explore patterns of similarity/dissimilarity in a sample with regard to several variables without
97 the need of *a priori* assumptions. Sperduti (1997) and Robb (1998) were the first to apply a
98 multivariate approach to the study of EC. Other examples of alternative statistical approaches
99 to the study of EC include the use of principal component analysis by Porčić and Stefanović
100 (2009), Stefanović and Porčić (2013), and Takigawa (2014), and the use of generalized
101 estimating equations by Villotte (2009) and Villotte and colleagues (2010).

102 In this study, we attempt to categorize occupations on the basis of a multivariate
103 analysis of EC using a large (N=372) identified human skeletal sample. The aim of the study
104 is to explore possible patterns of similarities in EC between occupations and to test if the
105 same patterns represent a valid basis for classifying occupations.

106 To do so, we postulate the following hypotheses:

107 H1) Assuming that EC, when controlling for age, reflect biomechanical and social
108 differences among occupations, we postulate that distinct groups of documented occupations
109 should be reflected by specific patterns of EC;

110 H2) If H1 is confirmed, we postulate that the identified subgroups share consistencies
111 from a biomechanical and/or social point of view, described through documented data
112 obtained through bibliographic research.

113

114

MATERIAL AND METHODS

115 The sample consists of 372 male individuals with known occupation from one Italian
116 identified human skeletal collection: the Frassetto collection of Sassari (Sardinia) (SISC),
117 housed at the Museum of Anthropology of the University of Bologna (N= 136, Milella et al.
118 2012); and from two merged Portuguese collections (Table 1). The identified human skeletal

119 collection of Coimbra (CISC), housed at the Department of Life Sciences in Coimbra
120 University (Rocha, 1995) (former Department of Anthropology), and the Luis Lopes
121 Collection (LLISC) (Cardoso 2006) housed at the Museum of Natural History in Lisbon (N =
122 236) were considered as a unique sample due to their similar chronological, historical, social
123 and cultural settings (Alves Cardoso, 2008).

124 The major rationale for comparing these identified human skeletal collections (IHSC)
125 is their chronological and cultural consistency. Both the Italian and the Portuguese collection
126 represent pre-industrial (beginning of the 20th century) societies and are marked by an overlap
127 regarding the documented occupations.

128 Only males were selected for analysis due to the scarce and ambiguous information on
129 female's occupation. Female individuals in all considered collections are indeed mostly
130 classified as "housewives", a term that, due to its generality, unfortunately hampers an
131 exploration of the possible relationship between EC and occupation in females.

132 We expect that, even allowing for population differences, their individual signals
133 should not be strong enough to consistently bias our analyses. Specimens were chosen on the
134 basis of the following criteria: (i) age at death ≥ 20 years of age; (ii) absence of pathologies
135 possibly linked to extra-spinal enthesopathy formation (e.g. DISH) (Freemont 2002; Jurmain
136 1999; Martin-Dupont et al. 2006; Rogers and Waldron 1995); and (iii) absence of skeletal
137 changes possibly linked to altered body biomechanics (fractures, dislocations, and dysplasias).
138 The cutoff point of 20 years of age-at-death was selected so that the sample would be
139 representative of biologically mature adults, with almost all epiphyses fused.

140 Data on EC were originally collected by Milella and colleagues on SISC (Milella et al.
141 2012) and by Alves Cardoso on CISC and LLISC (Alves Cardoso 2008). In order to allow
142 comparison between datasets, we considered only data on enthesal surface rugosity -
143 "robusticity" - *sensu* Mariotti et al. (2007), and Hawkey and Merbs (1995). These changes

144 were originally scored by Milella and colleagues (2012) applying five degrees (from 0 to 4),
145 following Mariotti et al. (2007), and by Alves Cardoso (2008) applying four degrees (from 0
146 to 3) according to Hawkey and Merbs (1995). In a second step the original scores were then
147 converted into binary data, applying specific criteria to each dataset according to their
148 different theoretical backgrounds (Table S1). Data from Milella and colleagues (2012) were
149 classified as absence (0) and presence (1). Absence would include the original grades 0 and 1,
150 while presence the grades 2, 3, and 4. A different conversion was used for the data collected
151 by Alves Cardoso (2008), which considered enthesis type (fibrous vs. fibrocartilaginous).
152 Accordingly, absence (0) of a fibrous enthesis corresponds to the original grades 0 and 1, and
153 in fibrocartilaginous entheses to the grade 0. Presence (1) in fibrous entheses corresponds to
154 the grades 2 and 3, while in fibrocartilaginous entheses to the grades 1, 2, and 3. The criterion
155 underlying the different conversion of fibrous and fibrocartilaginous sites is based on their
156 distinct skeletal morphology, which, in the first case, is represented by smooth areas, while in
157 fibrocartilaginous sites by rough surfaces. The described strategy, which dichotomizes
158 robusticity development, was chosen since it minimizes the bias introduced by differences in
159 the used scoring methods, allowing therefore a better (though admittedly not perfect)
160 comparability between observations.

161 Only sites analysed by both Milella and colleagues (2012) and Alves Cardoso (2008)
162 were considered. This led to a total of nine postcranial entheses, analysed by considering the
163 two sides separately (Table 2). In the case of the costoclavicular ligament, a distinction
164 between fibrous vs. fibrocartilaginous histology is not possible. Accordingly, the authors
165 decided to apply in this case the same criteria adopted for fibrocartilaginous sites. This choice
166 was dictated by the morphological variability of this site, which, besides some obvious
167 differences, can be compared with what is usually observed in fibrocartilaginous entheses.
168 Note that, in order to allow a comparison between the two datasets, only variables recorded by

169 both authors were considered in this study (i.e. enthesal robusticity, profession at death, and
170 age at death). Accordingly, relevant factors (e.g. body size, body mass) were excluded from
171 the analyses.

172 In order to check for the effect of asymmetry in robusticity, we also calculated an
173 asymmetry index by subtracting the left side from the right. Accordingly, the index can
174 assume the values 1, 0, and -1, reflecting right side dominance, lack of asymmetry, and left
175 side dominance, respectively. Due to their nonmetric nature, our enthesal scores cannot be
176 analysed through classical PCA. Accordingly, we used nonlinear principal component
177 analysis (NLPCA - Gifi 1990), by specifying a number of dimensions equal to the number of
178 variables (i.e. 18 for the full dataset, 9 for the asymmetry, left side, and right side datasets).
179 NLPCA is computed as an extension of simple homogeneity analysis after setting rank
180 constraints. Missing data are automatically treated according to the missing data passive
181 option (Gifi 1990), which discards missing observations from the overall computation (for a
182 full description see De Leeuw and Mair 2007, 2009). A hierarchical cluster analysis (by
183 Ward's minimum variance method) was then used to explore possible patterns in the datasets.
184 The obtained clusters were subsequently used as the basis for the classification of
185 occupations, and differences between occupation classes tested by means of MANOVA and
186 pairwise Hotelling's tests.

187 Due to the known effect of age on enthesal robusticity (Alves Cardoso and
188 Henderson 2010, 2013; Mariotti et al. 2007; Milella et al. 2012), age-at-death deviations from
189 normality were tested in all subsamples with the Shapiro-Wilk test. The latter was calculated
190 in order to assess any possible bias of the sample with relation to age. Different statistical
191 protocols are suggested in the literature to control for age. These include the use of age as
192 continuous explanatory variable in generalized estimating equations (Villotte 2009; Villotte et
193 al. 2010), subdivision of a sample in relatively small age classes (Milella et al. 2012),

194 comparisons of residuals from age-skeletal features regressions (Pinhasi et al. 2014). In the
195 present investigation, it was decided not to consider age categories due to the relatively small
196 size of specific professional groups and for the possible bias represented by categories with
197 different mean ages. On the other hand, both generalized estimating equations and residual
198 analysis were excluded in order not to lose potentially useful information in the multivariate
199 analyses. Consequently, a third strategy was adopted, which tests the correlation between age
200 and each principal component (PC) after computing NLPCA, excluding in the follow-up
201 analyses the PC(s) which are significantly correlated with age (choosing as threshold
202 Pearson's r absolute values ≥ 0.2).

203 Given the possible bias introduced in our multivariate analyses by the small sample
204 size of some occupations (Table 1), we repeated all procedures a second time, by considering
205 only occupations with a sample size equal or superior than five.

206 NLPCA was computed with the package *homals* (De Leeuw and Mair 2007, 2009) in the
207 software R version 3.0.2 (R Core Team 2014). Shapiro-Wilk test, Pearson's r test, and
208 hierarchical cluster analysis were calculated with JMP®10.0 (SAS Institute Inc. 2012).
209 MANOVA and pairwise Hotelling's test were computed in PAST (Hammer et al. 2001). For
210 all tests, alpha was set at 95%.

211

212

RESULTS

213 *Age:* Age distribution deviates from the normal assumption in both the Italian and Portuguese
214 samples (Table S2). Results demonstrate that for all datasets apart from that of asymmetry,
215 the first PC (PC1) is the dimension characterized by the highest significant correlation with
216 age (Table 3). In the full dataset, a significant correlation with age is also shown by PC7.
217 Accordingly, in order to control for age in the subsequent analyses, we decided to exclude

218 PC1 from all analyses on the left, right and full datasets, together with PC7 for the latter. The
219 reader should be aware of this when finding references throughout the text to analyses
220 performed with “all PCs”. Results obtained from a correlation test between age and PCs after
221 excluding from the sample those occupations characterized by N lower than 5 (but
222 nonetheless opting for the inclusion of the Portuguese farmers (N=3) in order to check for
223 their positioning in this alternative dataset) are consistent with what was observed in the
224 complete dataset. In this case, PCs showing a significant correlation with age and therefore
225 excluded from the subsequent analyses are PC1 (left, right, both sides), PC3 (asymmetry), and
226 PC6 (both sides).

227 *Exploratory multivariate analysis:* A cluster analysis performed on the PCs of the full dataset
228 (after excluding PC1 and PC7 due to their significant correlation with age) fails to show a
229 clear separation between the Italian and the Portuguese samples. Individuals from the two
230 groups fail to form two different clusters (Figure1). This result confirms our initial hypothesis
231 about a low population signal in the overall EC data, therefore justifying the pooling of the
232 datasets from the various collections. Cluster analyses of all PCs (after excluding from each
233 dataset the ones showing a significant correlation with age – see Methods section) highlights
234 complex patterns in all datasets, consisting in several clusters grouping relatively highly
235 diversified occupations. A straightforward interpretation of these results is accordingly
236 difficult, given the apparent lack of social and/or biomechanical consistency in most of the
237 single clusters. Nonetheless, when considering the overall clusters, it is possible to recognize
238 a common pattern, represented by a relative closeness between occupations sharing the
239 following basic features: 1) occupations related to farming activities and rural context (e.g.
240 farmer, laborer); 2) occupations sharing relatively intense physical activity but not related to
241 farming (e.g. tinsmith, shoemaker), nor to a rural context; 3) occupations not featuring manual

242 or generalized physical tasks, not related to farming and at least in part referable to a more
243 urban contexts (e.g. lawyer, bank clerk, shop assistant).

244 This pattern is consistent throughout the complete, left side, and right side datasets. On
245 the other hand, cluster analyses of the asymmetry dataset do not evidence a specific
246 distribution of occupations (Figures 2-5).

247 *Test for differences between occupational categories:* Results from the cluster analysis
248 suggested the inclusion of occupations in three main classes: Class 1 (occupations related to
249 farming), Class 2 (physically demanding occupations not related to farming), and Class 3
250 (physically undemanding occupations). Note that in this way the criterion used for grouping
251 occupations is directly linked to EC patterns, therefore minimizing (though not eliminating,
252 see discussion) the bias introduced by *a priori* biomechanical, social, and cultural criteria.

253 In order to test the consistency of such groupings, we then compared them by means
254 of MANOVA and pairwise Hotelling's test, by using as variables in each dataset all principal
255 components after excluding the ones significantly correlated with age (PC1 for left, right, both
256 sides, PC3 for asymmetry, and PC6 for both sides).

257 Results reflect a complex scenario, with no differences between classes when
258 considering asymmetry, a consistent significant difference between Classes 1 and 3 in the
259 other datasets and a difference between Classes 2 and 3 when considering two sides together
260 and the right side only (Table 4a).

261 *Reduced dataset*

262 Results from the multivariate analyses on the dataset after excluding occupations with $N < 5$
263 are largely overlapping what observed in the complete sample. The cluster analysis highlights
264 indeed a distribution of occupations which is consistent with the criteria underlying Classes 1,

265 2, and 3 (Figures S1-4). This result is further confirmed by the MANOVA and Hotelling's
266 tests. Also in this case no difference emerges between classes in the asymmetry dataset,
267 whereas Classes 1 and 3 are different when considering the two sides together and left and
268 right sides separately. Classes 1 and 2 differ only for the left side (Table 4b).

269

270

271

DISCUSSION AND CONCLUSION

272 Any attempt to explore and compare the impact of "specific" occupations in human
273 skeletal remains based on the study of EC is considered a challenging endeavor. Theoretical,
274 methodological and interpretative constraints are normally advanced as major sources of bias
275 not only in the study of archaeological samples, but also in IHSC-based studies (Alves
276 Cardoso 2008; Alves Cardoso and Henderson 2010; Milella 2010; Milella et al. 2012). In
277 order to tackle these issues we explored the usefulness of an alternative approach for
278 exploring differences among documented occupations on the basis of a multivariate study of
279 EC patterns. Specifically, the aim of this work was to test the following hypotheses: H1)
280 distinct groups of documented occupations should be reflected by specific patterns of EC; and
281 H2) if H1 was confirmed, identified subgroups would share consistencies from a
282 biomechanical and/or social point of view.

283 Our results confirmed these hypotheses only partially by showing a separation
284 between individuals involved in farming activities (Class 1), subjects performing heavy
285 physical tasks not related to farming (Class 2), and subjects theoretically featuring a more
286 sedentary lifestyle (Class 3). Note however that such differences are not consistent throughout
287 all datasets (sides separately, sides pooled, asymmetry). In particular, differences between
288 Classes 2 and 3 and Classes 1 and 2 are found only in some instances.

289 It is interesting to note the lack of differences between classes when considering levels
290 of bilateral asymmetry. Patterns of bilateral asymmetry of EC should indeed represent a good
291 proxy of differences in physical activity (see e.g. Villotte and Knüsel 2014). In our case, it is
292 possible that differences between occupations are masked by the type of EC considered in this
293 study (robusticity), by the binary nature of our data (probably not able to capture subtle
294 differences between the sides), and the chosen statistical procedure (which is based on the
295 simultaneous analysis of asymmetry values from different attachment sites).

296 Regarding the differences between classes, the more consistent result is the contrast
297 between Classes 1 and 3. Such result is consistent with previous works on EC (e.g. Niinimäki
298 2011; Villotte et al. 2010), as well as with historical and ethnographical data (see Alves
299 Cardoso, 2008 for details).

300 The difference between Classes 1 and 3 can be related to both biomechanical as well
301 as physiological factors. From a biomechanical point of view, occupations related to farming
302 are likely to share high levels of generalized exposure to biomechanical stress and a
303 prolonged (and probably precocious) involvement of the subject in the same occupation
304 through time. In the Portuguese ethnographic literature, for example, farming activities
305 performed by the digger/ditcher (*cavador*) are described as one of most physically demanding
306 (Almeida and Martins, 2002). Furthermore, historical evidence supports that many farming
307 activities started early in the individuals' lives. Statistical reports from the end of the 19th
308 century state that juvenile work was common and occurred in higher frequencies in some
309 Portuguese farming sectors. For instance, it is reported that between the years of 1870-1890
310 juvenile (and female) work increased by 700% in the Herdade de Palma – a farm dedicated to
311 extensive agriculture (Southern Portugal), and for farming tasks such as “*monda*” (picking
312 weeds in the rice fields) (Martins, 1997).

313 Moreover, farming activities are likely to share a relatively narrow range of
314 biomechanical stimuli (i.e. daily physical tasks). Overall, these features would contrast with
315 what one would expect from the wide range of occupations of the Class 3. Furthermore, it is
316 possible to postulate for Classes 2 and 3 a higher occupational mobility (i.e. transition during
317 life between different occupations) which would result in a more differentiated lifestyle, a
318 point consistent with the lack of differences between Class 2 and 3. The only partial contrast
319 evidenced between Class 1 and 2 can be interpreted as the byproduct of the high levels of
320 biomechanical stress characterizing the occupations included in these groups, which would
321 obscure more nuanced differences in terms of specialization of activity patterns (e.g.
322 lateralization). On the other hand, the homogeneity characterizing the professions included in
323 Class 1 (as opposed to the higher variance of professions included in Class 2) can be
324 considered also in this case an important factor contributing to the observed differences.

325 An interesting example of the homogeneity apparently characterizing occupations of
326 Class 1 is the proximity of farmers and workers in the Portuguese sample. Individuals
327 ascribed to these categories would have been involved in a significant number of various
328 activities during their lifetime, many of which sharing similar movements. They could,
329 therefore be described as people performing a relatively narrow and uniform range of tasks. In
330 a broad sense, farmers can be defined as subjects involved in working the land or more
331 generally involved in agricultural activity. On the other hand, workers are described as
332 people that would perform various tasks and activities related to farming, as well as
333 conducting other types of work not only in rural but also in urban settings. However, these
334 categories are not mutually exclusive. In Portugal, for example, many occupational categories
335 fall into the farming group, such as the previously mentioned ditcher/digger (*cavador*). In
336 contexts involving extensive farming economies, the ditcher belonged to the group of workers
337 that performed any kind of job with the hoe and in any given season (Almeida and Martins

338 2002), while the daily-laborer/journey-man (*jornaleiro*) is described as a simple, wage-rural
339 worker that performed any type of task related to farming, such as digging, sowing or reaping
340 (Almeida 2002). Moreover, many farmers were also acting as workers (Alves Cardoso 2008).
341 Hence, both farmers and workers would have been consistently exposed to hard physical
342 labour during their lifetime, contrasting with other occupations that would either be
343 specialized or featuring different physical demands.

344 Apart from biomechanical factors, it is worth considering the possible relevance of
345 physiological factors on the expression of EC. These would include genetic, hormonal, and
346 dietary factors. While genetic as well as hormonal variables are involved in the ontogeny,
347 maintenance, and rate and type of degeneration of the musculo-skeletal system (Atteno et al.
348 2014; Karasik and Kiel 2010; Liang et al. 2009; Pocock et al. 1987; Smith et al. 1973; Smith
349 and Smith 2002), such factors are unlikely to greatly influence the results of our study, due to
350 the composition of our sample which include only one sex, no distinct pedigree-based clusters
351 and the absence of subjects affected by (at least obvious) genetic or hormonal-based skeletal
352 disorders. On the other hand, differences in diet, though not tested in this study, are worth to
353 be considered as a possible factor influencing our results (see also Alves Cardoso and
354 Henderson 2010; Milella 2010). Diet, especially regarding the relative intake of proteins,
355 calcium, phosphorus and vitamin D, greatly influences variables like skeletal muscle mass,
356 bone mass, bone mineral density and bone mineral content (e.g. Deutz et al. 2014; Dideriksen
357 et al. 2013; Ilich et al. 2003; New 2002; Rosen 2002; Seibel 2007; Vicente-Rodriguez et al.
358 2008). Considering entheses as the interface between the muscular and skeletal system, we
359 hypothesize that their variability could, at least in part, be influenced by differences in dietary
360 regimes deriving from the socio-economic variability characterizing our sample. Note
361 however that, for the moment, discussing an influence of different diet regimes on enthesal
362 changes remains only an interesting working hypothesis.

363 Of particular relevance is the argument regarding the following challenges associated
364 with the current investigation: 1) the inclusion in the same analyses of different types of
365 entheses; 2) the analysis of only one type of EC (specifically robusticity), on the basis of data
366 collected by different observers using different methodologies; and 3) the lack of specific
367 sociocultural and biomechanical information on a large part of the occupations represented in
368 our sample. The first two issues derived from the need to maximize the sample size of each
369 occupation and the number of variables, at the same time trying to minimize the possible bias
370 represented by the use of different methodologies. Accordingly, our results, though
371 promising, can be interpreted only as a preliminary test of general enthesal changes (here
372 robusticity). It is possible that more specific results would be obtained by using a larger
373 number of attachment sites and controlling for their specific anatomy (e.g. by conducting
374 multivariate analyses separately for fibrous and fibrocartilaginous sites), as well as by
375 including in the analyses additional types of EC, ideally recorded by the same observer, or, at
376 least, by different observers using the same scoring method. In particular, the inclusion in the
377 analyses of data on enthesopathies would allow a discussion of results on the basis of data
378 from clinical and anatomical studies. Concerning the third issue, the lack of detailed
379 information on the life-style characterizing most of the occupations discussed in this study
380 represent an obvious (and likely unavoidable) limit when discussing our results. Furthermore,
381 due to the type of available documentation (which report the occupation performed by a
382 subject around the time of death), factors like occupations and in general activities performed
383 during the entire life course, as well as simultaneously to the documented one cannot be
384 considered in the discussion of results. The type of available documentation on each
385 profession is also a limit when trying to avoid *a priori* criteria in the classifications of
386 occupations. It should be noted that, while our classification of subjects is primarily dictated
387 by their relative proximity in the obtained clusters, the interpretation of the latter is also

388 influenced by *a priori* biomechanical hypotheses. The resulting subjectivity could be one of
389 the factors underlying the ambiguous pattern of differences between Classes 1, 2 and 3
390 evidenced by our analyses.

391 Despite such technical and theoretical considerations, the present work was
392 nonetheless able to identify important basic patterns in our sample, possibly correlated to
393 relevant biomechanical and sociocultural factors. On the other hand, it demonstrates the
394 usefulness of a multivariate approach to the study of EC, and, more in general, the advantage
395 of a research design not constrained by *a priori* assumptions in testing biocultural hypotheses.

396

397

398

399

ACKNOWLEDGEMENTS

400 We thank the editor-in-chief, the associate editor and the two anonymous reviewers
401 for detailed comments, which helped improve this manuscript.

402 The authors would like to thank the organisers, S. Villotte, C. Henderson, F. Alves
403 Cardoso and G. Perréard-Lopreno, of the Wenner-Gren Foundation funded (grant number Gr.
404 CONF-632) workshop entitled "Enteseal Changes and Reconstruction of Human Behavior:
405 Towards Standardization" hosted by CIAS (Research Centre for Anthropology and Health,
406 University of Coimbra, Portugal) with support from CRIA (Centro em Rede de Investigação
407 em Antropologia, Universidade de Nova, Lisbon, Portugal). We wish to extend our thanks to
408 the members of the other working groups for their contributions to our discussions: C.
409 Henderson, R. Jurmain, V. Mariotti, D. Pany-Kucera, S. Villotte and C. Wilczak. Thanks also
410 the student volunteer, Joana Dinis, who provided assistance throughout the workshop. Finally,
411 we would like to thanks the collections curators, and respective institutions for allowing
412 access to the identified skeletal collection. Francisca Alves Cardoso was supported by FCT

413 through postdoctoral research grant (SFRH/BPD/43330/2008) and Short-term Research Grant
414 awarded by the Calouste Gulbenkian Foundation (Grant n. 90106).

415

416

417

418

ABBREVIATIONS

419 IHSC = Identified human skeletal collections

420 SISC = Frassetto identified skeletal collection of Sassari

421 CISC = Identified human skeletal collection of

422 LLISC = Luis Lopes identified skeletal collection

423 NLPCA = Nonlinear principal component analysis

POST PRINT VERSION

- 425 Almeida MAPD. 2002. Jornaleiro. In: Martins CA, and Monteiro NG, editors. História
426 do Trabalho e das Ocupações. Oeiras: Celta Editora. p 204-216.
- 427 Almeida MAPD, and Martins CA. 2002. Cavador. In: Martins CA, and Monteiro NG,
428 editors. História do Trabalho e das Ocupações. Oeiras: Celta Editora. p 153-155.
- 429 Alves Cardoso F. 2008. A Portrait of Gender in Two 19th and 20th Century Portuguese
430 Populations: A Palaeopathological Prespective. Durham: University of Durham.
- 431 Alves Cardoso F, and Henderson C. 2010. Enthesopathy Formation in the Humerus:
432 Data from Known Age-at-Death and Known Occupation Skeletal Collections.
433 *Am J Phys Anthropol*(141):550-560.
- 434 Alves Cardoso F, and Henderson C. 2013. The Categorisation of Occupation in
435 Identified Skeletal Collections: A Source of Bias? *Int J Osteoarch* 23(2):186-
436 196.
- 437 Atteno M, Costa L, Cozzolino A, Tortora R, Caso F, Del Puente A, Cantarini L, Scarpa
438 R, and Ciacci C. 2014. The enthesopathy of celiac patients: effects of gluten-free
439 diet. *Clin Rheumatol* 33(4):537-541.
- 440 Belcastro G, Rastelli E, Mariotti V, Consiglio C, Facchini F, and Bonfiglioli B. 2007.
441 Continuity or discontinuity of the life-style in Central Italy during the Roman
442 Imperial age-early Middle ages transition: diet, health, and behavior. *Am J Phys*
443 *Anthropol* 132:381-394.
- 444 Benjamin M, Evans E, and Copp L. 1986. The histology of tendon attachments to bone
445 in man. *J Anat* 149:89.
- 446 Cardoso HFV. 2006. Brief communication: The collection of identified human
447 skeletons housed at the Bocage Museum (National Nuseum of Natural History),
448 Lisbon, Portugal. *Am J Phys Anthropol* 129(2):173-176.
- 449 De Leeuw J, and Mair P. 2007. Homogeneity Analysis in R: The package homals.
450 Department of Statistics, UCLA.
- 451 De Leeuw J, and Mair P. 2009. Gifi methods for optimal scaling in R: The package
452 homals. *Journal of Statistical Software*, forthcoming:1-30.
- 453 Deutz NE, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, Cederholm T,
454 Cruz-Jentoft A, Krznarić Z, and Nair KS. 2014. Protein intake and exercise for
455 optimal muscle function with aging: Recommendations from the ESPEN Expert
456 Group. *Clin Nutr*.
- 457 Dideriksen K, Reitelseder S, and Holm L. 2013. Influence of amino acids, dietary
458 protein, and physical activity on muscle mass development in humans. *Nutrients*
459 5(3):852-876.
- 460 Freemont AJ. 2002. Mini-symposium: Non-neoplastic osteoarticular pathology.
461 Enthesopathies. *Curr Diagn Pathol* 8:1-10.
- 462 Gifi A. 1990. *Nonlinear Multivariate Analysis*: Wiley.
- 463 Hammer Ø, Harper D, and Ryan P. 2001. Past: Paleontological Statistics Software
464 Package for education and data analysis. *Paleontología Electrónica* 4: 1-9.
465 URL:< http://palaeo-electronica.org/2001_1/past/issue1_01.html.
- 466 Henderson CY, Craps DD, Caffell AC, Millard AR, and Gowland R. 2013.
467 Occupational Mobility in 19th Century Rural England: The Interpretation of
468 Enteseal Changes. *Int J Osteoarch* 23(2):197-210.
- 469 Ilich JZ, Brownbill RA, and Tamborini L. 2003. Bone and nutrition in elderly women:
470 protein, energy, and calcium as main determinants of bone mineral density.
471 *European journal of clinical nutrition* 57(4):554-565.

- 472 Jurmain R. 1999. *Stories from the Skeleton. Behavioral Reconstruction in Human*
473 *Osteology*. Australia: Gordon and Breach Publishers.
- 474 Jurmain R, Alves Cardoso F, Henderson C, and Villotte S. 2012. Bioarchaeology's Holy
475 grail: the reconstruction of activity. In: Grauer A, editor. *A companion to*
476 *paleopathology*. Oxford: Wiley - Blackwell p531-552.
- 477 Karasik D, and Kiel DP. 2010. Evidence for pleiotropic factors in genetics of the
478 musculoskeletal system. *Bone* 46(5):1226-1237.
- 479 Liang G, Katz LD, Insogna KL, Carpenter TO, and Macica CM. 2009. Survey of the
480 enthesopathy of X-linked hypophosphatemia and its characterization in Hyp
481 mice. *Calcif Tissue Int* 85(3):235-246.
- 482 Mariotti V, Facchini F, and Belcastro MG. 2004. Enthesopathies - Proposal of a
483 standardized scoring method and applications. *Coll Antropol* 28:145-159.
- 484 Mariotti V, Facchini F, and Belcastro MG. 2007. The study of entheses: proposal of a
485 standardised scoring method for twenty-three entheses of the postcranial
486 skeleton. *Coll Antropol* 31(1):291-313.
- 487 Martin-Dupont S, Cunha E, Rougé D, and Crubezy E. 2006. Spondylarthropathy
488 striking prevalence in a 19th–20th century Portuguese collection. *Joint Bone*
489 *Spine* 73:303-310.
- 490 Milella M. 2010. *Skeletal Markers of Activity: Methodological and Interpretative*
491 *Reflections after the study of the whole Frassetto Sassari identified skeletal*
492 *collection [PhD Thesis]: University of Bologna*.
- 493 Milella M, Belcastro MG, Zollikofer CPE, and Mariotti V. 2012. The effect of age, sex,
494 and physical activity on enthesal morphology in a contemporary Italian skeletal
495 collection. *Am J Phys Anthropol* 148(3):379-388.
- 496 New SA. 2002. Food groups and bone health. *Clin Rev Bone Miner Metab* 1(3-4):209-
497 218.
- 498 Niinimäki S. 2011. What do muscle marker ruggedness scores actually tell us? *Int J*
499 *Osteoarch* 21(3):292-299.
- 500 Niinimäki S, and Baiges Sotos L. 2013. The Relationship Between Intensity of Physical
501 Activity and Enteseal Changes on the Lower Limb. *Int J Osteoarch* 23(2):221-
502 228.
- 503 Ockert B, Braunstein V, Sprecher C, Shinohara Y, Kirchhoff C, and Milz S. 2012.
504 Attachment sites of the coracoclavicular ligaments are characterized by
505 fibrocartilage differentiation: a study on human cadaveric tissue. *Scand J Med*
506 *Sci Sports* 22(1):12-17.
- 507 Perréard Lopreno G, Alves Cardoso F, Assis S, Milella M, and Speith N. 2013.
508 Categorization of Occupation in Documented Skeletal Collections: Its
509 Relevance for the Interpretation of Activity-Related Osseous Changes. *Int J*
510 *Osteoarch*(23):175–185.
- 511 Pinhasi R, Timpson A, Thomas M, and Slaus M. 2014. Bone growth, limb proportions
512 and non-specific stress in archaeological populations from Croatia. *Annals of*
513 *human biology* 41(2):125-135.
- 514 Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, and Eberl S. 1987.
515 Genetic determinants of bone mass in adults. A twin study. *J Clin Invest*
516 80(3):706.
- 517 Porčić M, and Stefanović S. 2009. Physical activity and social status in Early
518 Bronze Age society: The Mokrin necropolis. *Journal of Anthropological*
519 *Archaeology* 28(3):259-273.

- 520 R Core Team. 2014. R: A language and environment for statistical computing. Vienna,
521 Austria.: R Foundation for Statistical Computing. Robb JE. 1998. The
522 interpretation of skeletal muscle sites: a statistical approach. *Int J Osteoarch*
523 8(5):363-377.
- 524 Rogers J, and Waldron T. 1995. *A Field Guide to Joint Diseases in Archaeology*. New
525 York: John Wiley.
- 526 Rosen CJ. 2002. Nutrition and bone health in the elderly. *Clin Rev Bone Miner Metab*
527 1(3-4):249-260.
- 528 Seibel MJ. 2007. Bone turnover in nutrition-related disorders. *Wiener medizinische*
529 *Wochenschrift* 157(23-24):582-588.
- 530 Smith DM, Nance WE, Kang KW, Christian JC, and Johnston Jr CC. 1973. Genetic
531 factors in determining bone mass. *J Clin Invest* 52(11):2800.
- 532 Smith FW, and Smith PA. 2002. Musculoskeletal differences between males and
533 females. *Sports Medicine and Arthroscopy Review* 10(1):98-100.
- 534 Sperduti A. 1997. Life conditions of a roman imperial age population: Occupational
535 stress markers and working activities in Lucus Feroniae (Rome, 1st–2nd cent.
536 AD). *Human evolution* 12(4):253-267.
- 537 Stefanović S, and Porčić M. 2013. Between-group Differences in the Patterning of
538 Musculo-skeletal Stress Markers: Avoiding Confounding Factors by Focusing
539 on Qualitative Aspects of Physical Activity. *Int J Osteoarch* 23(1):94-105.
- 540 Takigawa W. 2014. Age changes of musculoskeletal stress markers and their inter-
541 period comparisons. *Anthropological Science* 122(1):7-22.
- 542 Vicente-Rodriguez G, Ezquerro J, Mesana MI, Fernandez-Alvira JM, Rey-Lopez JP,
543 Casajus JA, and Moreno LA. 2008. Independent and combined effect of
544 nutrition and exercise on bone mass development. *Journal of bone and mineral*
545 *metabolism* 26(5):416-424.
- 546 Villotte S. 2009. *Enthésopathies et Activités des hommes préhistoriques. Recherche*
547 *méthodologique et application aux fossiles européens du paléolithique supérieur*
548 *et du mésolithique: Bar International Series*.
- 549 Villotte S, Castex D, Couallier V, Dutour O, Knüsel CJ, and Henry-Gambier D. 2010.
550 *Enthesopathies as Occupational Stress Markers: Evidence from the Upper Limb*. *Am J*
551 *Phys Anthropol*(142):224-234.
- 552 Villotte S, Knüsel CJ. 2014. "I sing of arms and of a man...": medial epicondylitis and
553 the sexual division of labour in prehistoric Europe." *J Archaeol Sci* 43: 168-174.

554

555

556

557

558

559 **Figure captions**

560

561 FIGURE 1. Cluster representing mean PCs distance matrix of the full dataset. Note the
562 lack of separation between the Italian and Portuguese samples (colored, respectively, in
563 black and grey).

564 FIGURE 2. Complete dataset, both sides: a) Cluster representing mean PCs distance
565 matrix (after excluding PC1 and PC7). Note the relative association between
566 occupations related to farming (Class 1, light grey circles), physically demanding
567 occupations (Class 2, dark grey triangles), and physically undemanding occupations
568 (Class 3, black inverted triangles).b) Cluster representing mean PCs distance matrix by
569 class.

570 FIGURE 3. Complete dataset, asymmetry scores: a) Cluster representing mean PCs
571 distance matrix of the asymmetry dataset. Note the apparently random distribution of
572 occupations. Class 1: light grey circles; Class 2: dark grey triangles; Class 3: black
573 inverted triangles. b) Cluster representing mean PCs distance matrix by class.

574 FIGURE S1. Reduced dataset, both sides: a) Cluster representing mean PCs distance
575 matrix (after excluding of PC1 and PC6) by including both sides. Note the relative
576 association between occupations related to farming (Class 1, light grey circles),
577 physically demanding occupations (Class 2, dark grey triangles), and physically
578 undemanding occupations (Class 3, black inverted triangles). b) Cluster representing
579 mean PCs distance matrix by class.

580 FIGURE S2. Reduced dataset, left side: a) Cluster representing mean PCs distance
581 matrix (after excluding of PC1). Note the relative association between occupations
582 related to farming (Class 1, light grey circles), physically demanding occupations (Class
583 2, dark grey triangles), and physically undemanding occupations (Class 3, black
584 inverted triangles). b) Cluster representing mean PCs distance matrix by class.

585

586 FIGURE S3. Reduced dataset, right side: a) Cluster representing mean PCs distance
587 matrix (after excluding of PC1). Note the relative association between occupations
588 related to farming (Class 1, light grey circles), physically demanding occupations (Class
589 2, dark grey triangles), and physically undemanding occupations (Class 3, black
590 inverted triangles). b) Cluster representing mean PCs distance matrix by class.

591

592 FIGURE S4. Reduced dataset, asymmetry scores: a) Cluster representing mean PCs
593 distance matrix (after excluding of PC3). Note the apparently random distribution of
594 occupations. Class 1: light grey circles; Class 2: dark grey triangles; Class 3: black
595 inverted triangles. b) Cluster representing mean PCs distance matrix by class.

596

POST PRINT VERSION