Comparison of the entheseal changes of the *os coxae* of Portuguese males (19th-20th centuries) with known occupation

Short title: Comparison of entheseal changes of male *os coxae*

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ABSTRACT

The possible association between entheseal changes and activity has been widely studied. However many questions remain. This study aims to assess if occupation and physical activity influences the age at which entheseal changes appear in the iliac crest, retroauricular area, iliac tuberosity, ischial tuberosity and *obturator foramen*. Absence or presence of ossification exostosis and stress lesions was recorded in *os coxae* from 130 males (19 to 88 years old) from Lisbon and Coimbra identified skeletal collections. The individuals were divided into two groups, based upon to the recorded occupations: manual (n = 69) and non-manual (n = 61). The sample was also divided according to an osteological indicator of physical activity: the femur robusticity index (55 are robust and 54 are gracile femora). The individuals from manual and robust groups were considered to have had physically demanding occupations, while the non-manual and gracile groups represent individuals with less demanding activities. The asymmetry of entheseal changes between left and right sides of the same individuals was tested with a Chi-square test. And the influence of occupation and physical activities on the age of appearance of entheseal changes were tested using logistic regression. Statistically significant asymmetry was not found between left and right bones ($p < 0.05$). However, for the logistic regression calculations the only valid result was obtained for the ossification exostosis on the iliac crest for the measure of femoral robusticity. For the iliac crest, physical activity did not influence the appearance of ossification exostosis. It was not possible to obtain valid logistic regression models, probably due to the distribution of individuals in each occupational and robusticity category. Therefore it was not possible to assess the influence of occupation and physical activity on the age at which entheseal changes appeared for retroauricular area, iliac tuberosity, ischial tuberosity and *obturator foramen*.

Key words: Markers of occupational stress; musculoskeletal stress markers; enthesopathy; robusticity

Introduction

The reconstruction of identity and lifestyle, including physical activity, is widely undertaken by those studying archaeological and forensic skeletal remains. Physical activity is normally assessed by analyzing markers of occupational stress (MOS), such as entheseal changes.

Entheseal changes (Jurmain and Villotte, 2010) are musculoskeletal markers (Hawkey and Merbs, 1995), possibly caused by entheseal inflammation or continuous stress (Jurmain, 1999; Mariotti *et al.*, 2004). As with all bone changes they can either be osteophytic or osteolytic (Mariotti *et al.*, 2004). Therefore, entheseal changes have been used as an osteological evidence of occupation in past communities (*e.g.* Kennedy, 1983; Dutour, 1986; Angel *et al.*, 1987; Hawkey and Merbs, 1995; Peterson, 1998; Steen and Lane, 1998; Al Oumaoui *et al.*, 2004; Józsa *et al.*, 2004; Molnar, 2006; Lieverse *et al.*, 2009). Nevertheless, some authors argue (*e.g.* Jurmain, 1999; Weiss, 2003; Cardoso, 2008; Alves Cardoso and Henderson, 2010; Jurmain *et al.*, 2012) that the connection between occupation and entheseal changes is not as direct as it was previously believed.

The possible association between entheseal changes and physical activity has been widely studied (Cunha and Umbelino, 1995; Jurmain, 1999; Mariotti *et al.*, 2004; 2007; Cardoso, 2008; Alves Cardoso and Henderson, 2010; Villotte *et al.*, 2010; Santos *et al.*, 2011; Milella *et al.*, 2012). However many questions remain, particularly for the entheseal changes of the *os coxae*, which is less frequently investigated (Cunha and Umbelino, 1995; Pálfi and Dutour, 1995; Robb, 1998; Villotte, 2006; Cardoso, 2008) than long bones. The existing studies of the entheseal changes in the *os coxae* focus on three main areas: the ischial tuberosity, the iliac crest and the iliac tuberosity. Therefore, it is necessary to perform a more systematic study using identified collections (Alves Cardoso and Henderson 2010; Mariotti et al. 2009).

Occupation and physical activity are two different concepts. Occupation refers to the individuals' professions stated in the collection's records, and physical activity the level of bone mechanical loading by physical actions, assessed by an osteological parameter, e.g. the femur robusticity. The hypothesis to be tested was that entheseal changes would appear earlier in those individuals with a more demanding occupation and physical activity. The present study aims to test this hypothesis on the *os coxae* of adult males from Portuguese identified skeletal collections.

Materials and Methods

The male individuals analyzed belong to two Portuguese identified skeletal collections, which include data on occupation, sex and age at death. Sixty seven skeletons are from the Department of Life Sciences, University of Coimbra (Rocha, 1995; Santos, 2000) and sixty three from the National Museum of Natural History, University of Lisbon (Cardoso, 2006). During the selection of the sample, individuals with pathological changes visible on the *os coxae* and possible cases of spondyloarthropathies and diffuse idiopathic skeletal hyperostosis (DISH) were excluded, according to the data provided by Francisca Alves Cardoso (personal communication) and Carina Marques (personal communication and in Marques, 2007). Women were not included because their occupations were mainly recorded as "*domésticas*", a word that includes housekeepers and housewives, not allowing the distinction between individuals who had a more or a less demanding occupation. Male individuals were selected in order to have a similar number of skeletons by age class in each occupation group.

From the 130 male individuals, 257 (98.9%) *os coxae* were studied and three were excluded due to poor preservation. These individuals have ages at death ranging from 19 to 88 years (Figure 1), with a mean age of 46.5 years old and standard deviation of 17.1 years.

According to the occupation stated in the collections' records the sample was divided into two groups: sixty one non-manual individuals (*e.g.* priest, student), with age at death from 20 to 88 years old (mean = 45 years; median = 43 years; standard deviation = 17.5 years), and sixty nine manual individuals (*e.g.* farmer, carpenter), with age at death from 19 to 79 years old (mean = 48 years; median = 47 years; standard deviation = 16.7 years). The division was made taking in consideration the texts from Armstrong (1972) and Cardoso (2008). As stated by Alves Cardoso and Henderson (2010), the division into manual and non-manual was an attempt to determine the possible association between the entheseal changes and general levels of activity.

The assumption was made that the manual group and robust individuals all had physically demanding occupations/activities, as opposed to the non-manual group and gracile individuals for whom the assumption was that the activities and occupations were less intense (Campanacho *et al.*, 2012). In the present study a distinction was made between the terms occupation and physical activity. Occupation refers to the professions reported and physical activity is based in an osteological measure of activity, the femur robusticity. The occupations recorded may only refer to the last occupation of the individual and may not correspond to *in vivo* physical strain. Also, the records may not be a reliable source (Armstrong, 1972; Vidal, 2004). Therefore, femoral robusticity was used as an independent measurement of the mechanical forces/stress involved in activities. The mechanical loading leads to remodeling in bone's shape and size (Wescott, 2008), and bone robusticity (Ruff, 2008). For this reason the sample was also divided according to an osteological indicator, the femoral robusticity index (FRI, Olivier and Demoulin, 1984). The FRI is calculated by the equation: (Perimeter or midshaft circumference / Maximum length) x 100 (described in Olivier and Demoulin, 1984). Measurements of the left femur (right femur was used when the left was unavailable) were collected respectively with tape and an osteometric board (for measurement descriptions see Buikstra and Ubelaker, 1994). Therefore, the sample was divided into: robust (n = 55; FRI ≥ 20.34; from 24 to 83 years old; mean = 49 years; median = 46 years; standard deviation = 14.9 years) and gracile (n = 54; FRI < 20.34; from 20 to 88 years old; mean = 43 years; median = 39 years; standard deviation = 16.7 years) where the cut-off point is the median value of FRI (20.34). The median value of FRI was chosen because of the non-normal distribution of data (kurtosis: 2.694; skewness: 0.725). Due to poor preservation and/or pathology only 109 femora (out of 130 or 83.8%) were included in the final sample.

There is not a total correspondence between the robusticity and the occupational groups for the 109 individuals (Table 1). Therefore the associations between occupation and entheseal changes, and robusticity and entheseal changes were tested separately. Due to differential preservation the number of individuals differ in the occupational and robusticity groups.

The absence or presence of ossification exostosis and stress lesions (cortex pitting that resembles a lytic lesion) (Hawkey and Merbs, 1995), at entheses were recorded separately in the following areas: iliac crest, retroauricular area, iliac tuberosity, ischial tuberosity and *obturator foramen*. Figure 2 demonstrates a normal enthesis, an ossification exostosis and a stress lesion. In the present study, as in Cardoso (2008), the entheseal changes were recorded in osteological areas instead of recording by the enthesis boundaries of ligaments and muscles. This procedure avoided the need to establish the proper enthesis limits and the error associated with an incorrect delineation. Table 2 lists the ligaments and the muscles that are attached in the studied areas and the number of individuals by each area analyzed along with the number of ossification exostosis and stress lesions observed for the total sample.

The intra-observer error of the recording method for entheseal changes was established by the number and percentage of different scores between the two observations of 20 left and right *os coxae* (from 13 individuals) two weeks after the first analysis. The intra-observer error for femoral measurements was calculated for 20 left femora using the technical error of measurement, the mean absolute difference and the coefficient of reliability (Ulijaszek and Kern, 1999).

The asymmetry of the presence of entheseal changes between the right and left *os coxae* from the same individual, by area, was tested using a Chi-square test for the total sample. This test was chosen to compare asymmetry in entheseal changes (e.g. whether they occur more frequently on one side than another) by individual.

Logistic regression was used to determine the age of transition (percentile 50 or median) from the stage absence to presence of entheseal changes for each occupational and robusticity group. The lower and upper quartiles as a measure of variability around the median were also calculated. The logistic regression model's validity was evaluated by the significance of the Wald statistic. Whenever a logistic regression model could not be successfully fitted to the data for a certain variable, due to the non-significance of Wald, the model was considered invalid and subsequently was eliminated from the analysis.

The evaluation of the existence of statistically significance differences between groups and to assess if the occupational and physical activity had some influence on entheseal changes was analysed using logistic regression by comparing the medians of the age of transitions. Thus, the median age was compared between manual and non-manual groups, and between robust and gracile individuals, treated as covariates. Outliers were considered to be two times superior to the standard deviation (Maroco, 2007). Outliers were removed during the logistic regression to test whether these affected the significance of the Wald statistic.

Chi-square test and logistic regression were performed in SPSS 17. It was considered to be statistically significant when $p<0.05$.

Results

Intra-observer error (Table 3) was low for all entheses except for the osteophytic changes on the ischial tuberosity. Therefore, ossification exostosis on the ischial tuberosity were excluded from the Chi-square and logistic regression analysis. For the femoral measurements the intra-observer error is low as show on Table 4.

No statistically significant difference was found in the asymmetry in entheseal changes between left and right *os coxae* for the same individual ($p < 0.05$). Thus, only the left innominate data was included in the logistic regression analysis (right innominate data was used when the left was unavailable). Due to the improved significance of the Wald statistic for the logistic regression model without the outliers than with the outliers, only the results obtained without the outliers are presented. Only the ossification exostosis on the iliac crest for the robust and gracile individuals presents valid logistic regression models, with a significant Wald. Table 5 presents the age of transition (median), the lower and upper quartiles for the robust and gracile individuals.

With the robusticity as a co-variate, the significance of Wald is 0.797, thus there is not a statistically significant difference in the age of appearance of ossification exostosis on the iliac crest between robust and gracile individuals.

For the others areas, from each occupational and robusticity groups, it was not possible to obtain valid logistic regression models, due to the non-significance of Wald. These non-valid results, are possibly due to the distribution of entheseal changes in the groups, for example: 1) stress lesions only occur in two individuals on the iliac crest, for the non-manual and gracile groups. On the ischial tuberosity occur in less than 10 individuals, for the occupation and robusticity groups; 2) ossification exostosis are present in the retroauricular area for all robust individuals, while in the gracile group is absent in 2 individuals; in the manual (n=2) and non-manual groups are absent only in 3 individuals; 3) stress lesions are absent in the *obturator foramen*, retroauricular area, and iliac tuberosity in occupation and robusticity groups, and for iliac crest for the manual and robust groups.

Discussion and Conclusion

The possible influence of occupation and physical activity on entheseal changes of the *os coxae* in documented male individuals from the 19th and 20th centuries are presented. This was assessed by the comparing the median age of entheseal changes between groups with different levels of physical demand. Only one valid sample could be tested using logistic regression: the ossification exostosis on the iliac crest for the robusticity groups. The valid result for the iliac crest was obtained by removing four outliers all mature adults ≥ 60 years old without an ossification exostosis in the iliac crest. In this sample, physical activity of the individuals does not seem to have influenced the development of ossification exostosis in the iliac crest. The median age of appearance of ossification exostoses is similar for robust and gracile individuals, occurring on the fourth decade of life, as shown in table 5. Thus, physical activity did not influence the appearance of entheseal changes. Studies of individuals of both sexes with known occupation found similar results. Cunha and Umbelino (1995) studied the association between occupation and entheseal changes in the post cranial skeleton from 151 individuals of both sexes from the Coimbra identified skeletal collection. The authors applied a different method (Crubézy 1988) to record entheseal changes at the iliac crest, the ischial tuberosity and the "bridge" between the ilium and sacrum. Their study revealed no association with occupation (Umbelino and Cunha, 2009). A study of a larger sample of the Lisbon and Coimbra identified skeletal collections found no association between occupation and entheseal changes of the iliac crest or ischial tuberosity (Cardoso, 2008)

The statistical non-significance of the influence of physical activity on the age of appearance of ossification exostoses on the iliac crest could be caused by the fact that entheseal changes are also influenced by other factors than occupation., e.g. diet, body size, locomotion, metabolism and pathologies such as DISH and spondyloarthropathies (Ball, 1971; Chadwick, 1989; McGonagle, 1998; Jurmain, 1999; Flemming *et al.*, 2003; Weiss, 2003; 2004; Martin-Dupont *et al.*, 2006; Marques, 2007; Cardoso, 2008). Although, possible cases of DISH and spondyloarthropathies were eliminated from the sample, no pathological analysis of the entire skeleton was performed. Consequently other pathologies may have influenced entheseal changes in the *os coxae*. Also, the reliability of the femoral robusticity as an osteological measure of physical activity must be questioned. However, due to the aforementioned problems associated with the occupational records this was chosen as another indicator of physical activity.The femur robusticity index measures bone deformation due to biomechanical stresses (Ruff, 2008). The external measurements may incorrectly express the femoral robusticity. Although, Wescott (2006) and Stock and Shaw (2007) argued that robusticity can be obtained by external measurements, but it is possible that the formula used was not appropriate to measure mechanical loading. Other factors also influence bone robusticity, such as age (Ruff and Hayes, 1983), pathologies (Brothwell and Browne, 2002) and diet (Sahni *et al.*, 2010) all of which may have had an impact on these results.

For the majority of areas analyzed, excepting the ossification exostosis of the iliac crest, no valid logistic regression models were obtained. Therefore, it was not possible to infer the influence of occupation and physical activity on the median age at which entheseal changes appeared. This could be the result of the distribution of the stages absence and presence of entheseal changes. For example, in some areas (Table 2) stress lesions were absent in all individuals. It could also be the result of the limitations associated with the collection records or a methodological problem.

Recorded occupation represents the last occupation and not the overall physical demands throughout life (Campanacho *et al.*, 2012). The biographic data for both collections offers the occupation of the individuals at death. It is unknown whether the individuals performed the same occupation all their lives or if it changed (Alves Cardoso and Henderson, 2010). Also, the reliability of the occupation recorded is unknown (Armstrong, 1972; Vidal, 2004) and as was stated by Henderson and co-authors (in press), in their study of a nineteenth century rural population, occupation recorded is not sufficiently informative to study entheseal changes. Categorization of occupations is difficult and as was recently demonstrated by Alves Cardoso and Henderson (in press) the results of a study can be influenced by the categories used to group the individuals. Another aspect can be associated with the fact that individuals placed in the non-manual and gracile groups may also have had demanding occupations and physical activities not recorded in the collections' files (Alves Cardoso and Henderson, 2010; Campanacho *et al.*, 2012). It is unknown if the individuals performed others activities outside their main work (Alves Cardoso and Henderson, 2010) and hierarchy within the same occupation may have existed since individuals can perform different tasks that involve different workload (Alves Cardoso and Henderson, 2010). However, these hypothetical situations are not among the data available for any of the Portuguese identified skeletal collections.

The authors also take into consideration the possibility that the recording method used for entheseal changes is not suitable. The methodology used (registration of presence and absent of ossification exostosis) is adapted from Hawkey and Merbs (1995), that ignores the new clinical information regarding entheses morphology and changes, particularly the two distinct morphologies (fibrous and fibrocartilaginous) of entheses (Villotte, 2006; Villotte and Knüsel, In press; Jurmain et al., 2012). Moreover, there is no standard scoring method available for the *os coxae*. The majority of methods were established without the analysis of the *os coxae*, e.g. Cruzéby (1988), Hawkey and Merbs (1995), Mariotti et al. (2004; 2007), Henderson et al., 2010; Villotte et al. (2010), the only exception is the scoring methods developed for the ischial tuberosity by Robb (1998) and Villotte (2006).

Few stress lesions were recorded in the overall sample. This might be due to the difficulty of differentiating normal porosity from lytic lesions. It is, therefore, possible that osteolytic lesions were underscored in this sample.

There were no statistically significant differences between the right and left *os coxae* from the same individual in the presence of entheseal changes. In contrast, Cardoso (2008) found a significant asymmetry of entheseal changes in the iliac crest in female individuals. Probably the difference obtained in these two studies results from the methodologies applied for entheseal changes recording.

This study analyzed the entheseal changes of *os coxae,* a skeletal region less frequently studied than the upper and lower limbs. From authors knowledge this was the first time that the possible influence of occupation and physical activity on the entheseal changes appearance in the *obturator foramen* area was assessed. As has been concluded in other studies the relationship between entheseal changes and individual activity is complex and requires further research using identified skeletal collections. The results of this paper do not support the hypothesis that entheseal changes occur earlier in those with physically more demanding occupations.

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Table 1. Distribution of the individuals by groups of occupation and robusticity.

Sample	**Robust group**		**Gracile group**		**Subtotal**
	N	**%**	**N**	**%**	**N**
Non-manual group	21	42.9	28	57.1	49
Manual group	34	56.7	26	43.3	60
Total	55	50.5	54	49.5	109

Table 2. Number of individuals (N) by ossification exostosis and stress lesions for the total sample and anatomical structures affected on each observed bone area.

Bone area	Ossification exostosis		Stress lesions			Anatomical structure
	Absence	Presence	Absence	Presence	Total	
Iliac crest	35	68	101	2	103	Gluteus maximus muscle Tensor fasciae latae muscle Iliocostalis and multifidus muscle Sartorius muscle Erector spinae muscle Transversus abdominis muscle Latissimus dorsi muscle Iliolumbar ligament Posterior sacrosciatic ligament
Retroauricular area	3	113	116	0	116	Iliocostalis and multifidus muscle
Iliac tuberosity	29	89	118	0	118	Sacroiliac interosseous ligament
Ischial tuberosity	45	74	108	11	119	Inferior gemellus muscle Quadratus femoris muscle Biceps femoris muscle Semitendinosus muscle Semimembranosus muscle Great adductor muscle Posterior sacrosciatic ligament
Obturator foramen	19	103	122	0	122	Obturador internus muscle Obturador externus muscle Obturator membrane

Table 3. Number and percentage of different scores between the two different observations (intra-observer error).

Bone area	**Type**	**N**	**%**
Iliac crest	Ossification exostosis	0	0
	Stress lesion	0	0
Retroauricular area	Ossification exostosis	0	0
Iliac tuberosity	Ossification exostosis	2	10
Ischial tuberosity	Ossification exostosis	5	25
	Stress lesion	0	0
Obturator foramen	Ossification exostosis	1	5

Table 4. Intra-observer results for the femoral maximum length and perimeter.

Calculations	Maximum length (mm)	Perimeter (mm)
Minimum	392	76
Maximum	503	99
Mean	438.95	87.15
Median	438	87
Technical error of measure	0.387	0.418
Coefficient of reliability	0.9998	0.9959
Mean average difference	0.100	0.150

Table 5. Transition age (median), lower quartile and upper quartile for the robust and gracile individuals for the ossification exostosis on the iliac crest.

Group	Lower quartile	Median	Upper quartile
Robust	25.0	34.4	43.9
Gracile	26.3	33.8	41.4

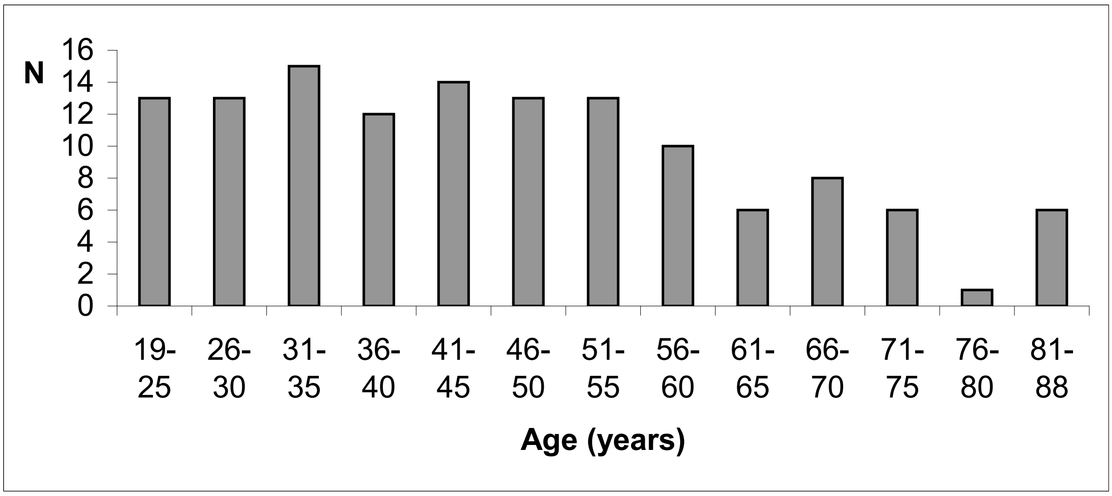

Figure 1. Distribution of the 130 male individuals by age at death range.

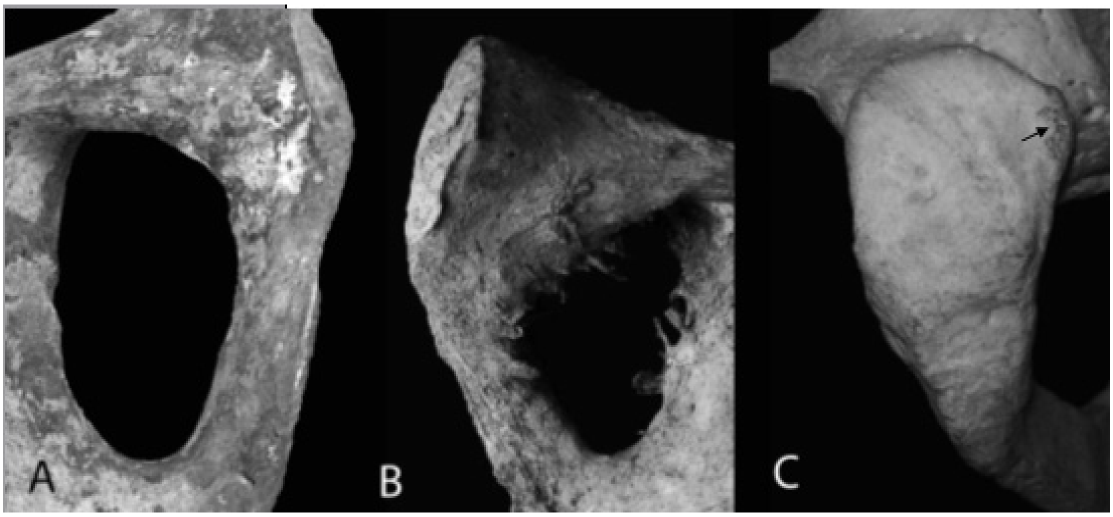

Figure 2. Image A represents a *obturator foramen* without entheseal changes. In contrast images B and C represents ossification exostosis and stress lesions, respectively.