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ARTICLE / *ARTICLE*

The New Coimbra Method for Recording Entheseal Changes and the Effect of Age-at-Death

La nouvelle méthode Coimbra : changement au niveau des enthèses et influence de l'âge au décès

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Abstract Entheseal changes have been widely used in anthropology to study activity patterns, but there is an increasing awareness that ageing is associated with these changes. The aim of this study was to test each feature of the new Coimbra method for its variability, side asymmetry and its relationship with age. In addition to this, an overall relationship with age was tested for a larger sample. Males 16 and over from the Coimbra skeletal collection of historically identified individuals were recorded using the new method (N = 260). To reduce the impact of occupation, side variability in asymmetry and age were only tested in the labourers (N = 51). All occupation groups were included to test the overall relationship with age using a random forest test. The results show that scores lack variability for many of the features and entheses. Where there is side asymmetry this is typically in favour of higher scores in the right side, excepting the biceps brachii insertion. Most of the features scored show a relationship with ageing, but this is not uniform for all features or entheses. Some features are associated with an increase in age (bone formation and erosions), while others generally occur in younger individuals (fine porosity and textural change). Logistic regression showed that ageing explains at most 44% of the variability. This alongside the side asymmetry may indicate that biomechanics has an explanatory role.

Keywords Asymmetry · Activity-patterns · Ageing · Degeneration

Résumé Les changements au niveau des enthèses ont été largement utilisés en anthropologie biologique pour discuter des patterns d'activités, malgré les études de plus en plus fréquentes associant ces changements principalement au vieillissement. L'objectif de cette étude est d'illustrer, pour chacune des modifications enregistrées avec la nouvelle méthode de Coimbra, la distribution générale des scores, l'asymétrie et leur relation à l'âge. Une étude plus globale sur l'effet du vieillissement a également été menée. L'analyse porte sur un échantillon de squelettes de sujets masculins décédés à 16 ans ou plus issus de la collection de squelettes identifiés de Coimbra (n = 260). Pour réduire l'influence de l'activité physique, seuls les sujets avec la profession de « trabalhador » (travailleur) ont été utilisés dans les tests sur l'asymétrie et l'âge (n = 51). Pour l'étude globale sur l'effet du vieillissement, toutes les professions ont été incluses dans une analyse utilisant les forêts aléatoires. Les résultats montrent que la variabilité des scores est faible pour la plupart des changements et des enthèses. Il existe une asymétrie assez claire avec des scores plus élevés du côté droit, sauf pour l'insertion du biceps brachii. La plupart des changements enregistrés présentent une corrélation positive avec l'âge au décès, sans toutefois être systématiques pour tous les changements ou toutes les enthèses considérées. Certains changements sont plus fréquents chez

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les sujets âgés (formation osseuse, érosion), alors que d'autres se retrouvent plus souvent chez les jeunes sujets (porosité fine et changement mineur de surface). Une régression logistique montre que le vieillissement explique au mieux 44 % de la variabilité perçue. Cela, ainsi que l'asymétrie directionnelle observée, pourrait indiquer que les phénomènes biomécaniques jouent un rôle dans l'apparition de ces changements.

Mots clés Asymétrie · Pattern d'activités · Vieillissement · Dégénération

Introduction

The most common method for identifying specific types of activities remains the observation and recording of entheseal changes (ECs) [1–3]. These are changes from the normal biological appearance of the attachment of tendons and ligaments to the bone [4]. They are frequently interpreted as indicators of repetitive loading of specific muscles, or muscle groups, and are therefore considered of good specificity to particular movements, which are then interpreted as tasks [3,5–7].

The enthesis itself is a poorly understood region of the muscle–bone complex, with limited current clinical research in this area. The research that exists focuses on changes associated with diseases, e.g. the seronegative spondyloarthropathies, which include diseases such as ankylosing spondylitis [8–11]. While the development of the enthesis and its normal appearance are well described in the literature [8,12], there has been little research on how the enthesis relates to its neighbouring structures, for example the interplay of tendon cross-sectional growth and enthesis size, or, tendon injury and response within the enthesis. The latter is of particular concern, as the inferences for using entheses as indicators of repetitive stress are reliant on a model based upon a direct interplay between tendon loading and enthesis damage.

What is clear from anthropological research is that ECs are associated with increasing age [13–22]. This has been shown for most recording methods when tested on identified skeletal collections (ibid.). It has also been used to support the argument that the changes represent cumulative repetitive stress, but this fails to take into account normal degeneration caused by increasing age, which is a cause of tendon tears [23]. Cumulative one-off trauma may also play a role, often in association with underuse because regular use promotes healthy tendon morphology [24]. The concept of underuse injury is beginning to be discussed in sports medicine where injuries often associated with high loads are found in those not accustomed to activity [25].

The new Coimbra method was developed from the original Coimbra method with the aim of creating a standardised scoring system for ECs while taking into account the normal biology and scoring a broad range of features seen at fibrocartilaginous entheses [16,26–27]. These entheses consist of four tissues of differing mechanical properties that mediate the transfer of stress from the tendon to the bone [8]. The method separates entheses into two zones based on the anatomy of the enthesis: i.e. zone 2 the most fibrocartilaginous part and zone 1, which is more fibrous running along the margin of the enthesis at the most obtuse angle of tendon attachment. Alongside this, it focuses on recording the types of changes seen macroscopically, "called features" that are textural change, bone formation, erosions, fine porosity, macroporosity and cavitations. The scoring consists of three scores (as well as a score for unobservable) for most features except textural change that is only scored as present or absent as it is hard to identify unless it covers a relatively large surface area.

The original method was tested on a sample of 31 individuals with similar occupations curated as part of the SIMON collection in Geneva [16]. It demonstrated first that left and right sides typically showed similar changes and that most of the changes were associated with ageing; although the small sample size and limited variability meant that the effects could not be fully interpreted. This paper repeats that approach using the new Coimbra method [26] on a larger sample of male skeletons, all with the same occupation. The aims are to test the relationship between ECs and age for each feature and determine bilateral asymmetry for ECs. To test the overall relationship between EC features and age a random forest approach was used, creating a prediction of age that could be compared to the real age, enabling the age effect to be determined.

Materials and methods

The Coimbra identified skeletal collection contains skeletal remains and documentary evidence of age-at-death, sex, occupation and cause of death for over 500 individuals who died in the early 20th century [28]. Diverse occupations are represented, but the predominant male occupation is 'trabalhador', meaning labourer. These individuals are likely to have predominantly worked the land as farm labourers undertaking seasonal work [29]. To fully capture age effects, male individuals aged from 16 upwards were included in this study. The minimum age of 16 was chosen because by this age all entheses examined in this study are fully developed and observable.

Several diseases, e.g. seronegative spondyloarthropathies and diffuse idiopathic skeletal hyperostosis, are widely described in the clinical literature as causing changes to the entheses [8–11]. To exclude individuals whose ECs may be pathological, all individuals with more than two vertebrae ankylosed by new osseous tissue along the line of the anterior longitudinal ligament were excluded from the analysis [30]. Individuals with ligamentous ankylosis of the sacroiliac joint with at least two vertebrae ankylosed were also excluded (ibid.).

The fibrocartilaginous entheses included in this analysis were the infra- and supraspinatus insertion, subscapularis insertion, common extensor origin, common flexor origin (all located on the humerus) and biceps brachii insertion (on the radius). All ECs were recorded using the new Coimbra method [26], although the biceps brachii insertion was recorded using the footprint published in the first version of the method [16]. The infra- and supraspinatus insertions were scored as one insertion because the fibres are known to merge in some areas close to the enthesis [31]. These entheses were all recorded by one observer (CH) with inter- and intraobserver error for this new method previously reported [32].

There are a total of six features recorded in the new Coimbra method [26]. Two features, bone formation [BF(Z1)] and erosion [ER(Z1)], are scored in zone 1. In zone 2, these features (labelled Z2) plus textural change (TC), fine porosity (FPO), macroporosity (MPO) and cavitations are scored. All, apart from textural change, are scored from 0 (absence of change) to 2 (maximal expression), while textural change is only scored as absence (0) or presence (1). The scoring has been simplified and standardised to three scores since the original method's publication [16] and textural change, which was scored as a type of bone formation, is now scored separately.

The variability of scores for each enthesis and feature is described, as it has been found that some features are more common at some entheses than others. Asymmetry of scores by enthesis and feature was calculated by counting the number of individuals with equal scores on both sides; with right side scores higher and with left side scores higher [16]. Frequencies for these results were also reported to enable comparison.

Ordinal regression was used to test the effect of age for each enthesis and feature within a single occupation group, as was done in the 2013 paper [16] to reduce potential biomechanical effects. The skeletons ($N = 51$) meeting the above criteria had an age range from 16 to 66 (mean = 41.29, standard deviation = 13.94). The Shapiro–Wilk test of normality showed a normal distribution of age-at-death $W = 0.96$, $P = 0.97$. To study the effect of age, means with standard errors were plotted for each enthesis, alongside descriptive statistics. Where there was sufficient variability, ordinal regression was performed using a cumulative link model [33] in *R* version 3.2.2. A log-log link was used, as in the previous study, because lower scores were more probable than higher ones [16]. Ordinal regression was performed for each feature, enthesis and side. Nagelkerke's pseudo-*R*-square was calculated to determine the effect of age using an *R* script [34]. Ordinal regression was only performed where there was variability of scores based on the presence of at least five occurrences of changes (score 1 or 2).

Previous analyses using this recording method have only tested each enthesis and feature individually for the effect of age. While it was demonstrated that each feature relates to age differently [16], no test has yet been performed using the variability from all entheses and all features simultaneously to study the impact of age. Our new approach is to create a prediction model of age, which can then be compared with true age to determine its effect. This could not be achieved with the labourers alone due to the relatively small sample size and its limited variability. For this study all males ($N = 260$) without signs of diseases associated with ECs (see above) were included. The age range was the same as for the ordinal regression study (mean = 44.8, standard deviation = 17.9). Random forests are a combination of regression trees and a bootstrap approach. Regression trees aim to partition the space into small homogeneous regions [35]. The trees are designed to build subsets with low within-variance and high between-variance, thereby partitioning the data according to an algorithm of rules. Thus, the algorithm will define some homogeneous regions of the feature space where the individuals have similar ages based on their overall EC expression. For random forests, several hundred regression trees are built, each of them using only a given portion of individuals and variables [36]. The overall prediction is obtained by taking the mean of the predictions of all trees. Random forests can handle ordinal predictors and have their own algorithm for missing value imputation. For this statistical analysis, the *R* package random forest was used [37]. The age of each individual was predicted using leave one out cross-validation. To determine the effect of age, the predicted age is plotted against the actual age such that if age is a major aetiology of these changes, the predicted age and the actual age should overlap, showing strong concordance.

Results

The overall distribution of changes (Table 1) demonstrates the low variability of scores with very few of the highest scores present. Only bone formation in zone 1 [BF(Z1)] shows a consistent presence of score 2 with the majority of individuals having scores of zero. No doubt this is also the reason most of the scores are equal between left and right sides (Table 2). Where there is asymmetry, the right side tends to have higher scores. It is noteworthy that the left side scores are more commonly higher than the right for all biceps brachii features.

Table 1 Descriptive statistics showing the variability of enthesis scores / *Statistiques descriptives illustrant la variabilité des scores*

Enthesis	Side	Enthesis score	BF(Z1)	ER(Z1)	BF(Z2)	FPO	MPO	ER(Z2)	CA	TC
Infra-an supraspinatus	Left	NA	17	17	8	8	8	8	8	8
		0	31	33	33	19	37	26	42	42
		1	3	1	9	21	6	16	1	1
		2	0	0	1	3	0	1	0	0
	Right	NA	16	16	9	9	10	10	9	9
		0	31	32	30	19	38	20	41	41
		1	3	3	10	22	2	17	1	1
		2	1	0	2	1	1	4	0	0
Subscapularis	Left	NA	4	4	4	4	4	4	4	4
		0	28	41	16	24	39	27	45	44
		1	12	6	26	23	8	19	2	3
		2	6	0	5	0	0	1	0	0
	Right	NA	9	9	5	5	5	5	5	5
		0	23	35	17	20	42	27	41	46
		1	13	6	24	25	4	18	5	0
		2	6	1	5	1	0	1	0	0
Common extensor origin	Left	NA	15	15	12	12	12	12	12	12
		0	30	36	35	37	39	33	39	39
		1	5	0	4	2	0	5	0	0
		2	1	0	0	0	0	1	0	0
	Right	NA	17	17	9	9	9	9	9	9
		0	23	34	29	37	41	34	42	42
		1	6	0	11	2	1	8	0	0
		2	5	0	2	3	0	0	0	0
Common flexor origin	Left	NA	18	18	13	13	13	13	13	13
		0	30	33	30	35	38	37	38	38
		1	3	0	6	3	0	1	0	0
		2	0	0	2	0	0	0	0	0
	Right	NA	20	20	15	14	14	14	14	14
		0	25	31	27	32	37	33	37	36
		1	4	0	7	5	0	4	0	1
		2	2	0	2	0	0	0	0	0
Biceps *brachii*	Left	NA	6	6	2	2	2	2	2	2
		0	25	43	30	34	44	44	48	31
		1	10	2	15	11	4	5	1	18
		2	10	0	4	4	0	0	0	0
	Right	NA	6	6	7	7	7	7	7	7
		0	26	45	33	31	43	42	44	34
		1	9	0	10	9	1	0	0	9
		2	10	0	1	4	0	2	0	1

Z1 refers to zone 1, Z2 to zone 2; BF: bone formation; ER: erosions; FPO: fine porosity; MPO: macroporosity; CA: cavitations; TC: textural change. For definitions, see Henderson et al. [26] / *Z1 correspond à zone 1, Z2 à zone 2 ; BF : formation osseuse ; ER : érosions ; FPO : porosité fine ; MPO : macroporosité ; CA : géodes ; TC : changement de texture. Pour les définitions, voir Henderson et al.* [26]

The effect of age typically showed an increase in mean age with higher scores for features, but this was not the case for FPO or TC where higher scores often occurred in younger individuals (Fig. 1). The limited variability of scores meant that ordinal regression could not be performed for all entheses and features. The feature with the most variable scores, bone formation both BF(Z1) and BF(Z2), had the most consistent statistical significance (at 95%) with age, but the pseudo-*R*-squared showed that the effect of age was typically minimal, at most explaining 44% of the variability

Table 2 Asymmetry scores for entheses by feature. Bold indicates a large number of higher scores on the left side / *Score d'asymétrie pour chaque enthèse, par type de changement. Une dominance à gauche est indiquée en gras*

Enthesis	Asymmetry	BF(Z1)		ER(Z1)		BF(Z2)		FPO		MPO		ER(Z2)		CA		TC	
		N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
Infra- and supra-spinatus	*N*	28		28		39		39		38		38		39		39	
	Right higher	1	3.6	0	0.0	6	15.4	8	20.5	2	5.3	9	23.7	1	2.6	1	2.6
	Equal	27	96.4	28	100.0	30	76.9	23	59.0	32	84.2	26	68.4	37	94.9	38	97.4
	Left higher	0	0.0	0	0.0	3	7.7	8	20.5	**4**	10.5	3	7.9	1	2.6	0	0.0
Subscapularis	*N*	42		42		45		45		45		45		45		45	
	Right higher	8	19.0	5	11.9	4	8.9	13	28.9	4	8.9	6	13.3	4	8.9	0	0.0
	Equal	30	71.4	34	81.0	35	77.8	23	51.1	33	73.3	31	68.9	40	88.9	42	93.3
	Left higher	4	9.5	3	7.1	**6**	13.3	9	20.0	**8**	17.8	**8**	17.8	1	2.2	**3**	6.7
Common extensor origin	*N*	31		31		34		34		34		34		34		34	
	Right higher	7	22.6	0	0.0	8	23.5	4	11.8	1	2.9	4	11.8	0	0.0	0	0.0
	Equal	24	77.4	31	100.0	25	73.5	28	82.4	33	97.1	26	76.5	34	100.0	34	100.0
	Left higher	0	0.0	0	0.0	1	2.9	2	5.9	0	0.0	4	11.8	0	0.0	0	0.0
Common flexor origin	*N*	25		25		31		32		32		32		32		32	
	Right higher	5	20.0	0	0.0	5	16.1	4	12.5	0	0.0	3	9.4	0	0.0	1	3.1
	Equal	17	68.0	25	100.0	21	67.7	26	81.3	32	100.0	29	90.6	32	100.0	31	96.9
	Left higher	3	12.0	0	0.0	5	16.1	2	6.3	0	0.0	0	0.0	0	0.0	0	0.0
Biceps b.	*N*	41		41		43		43		43		43		43		43	
	Right higher	7	17.1	0	0.0	4	9.3	7	16.3	0	0.0	2	4.7	0	0.0	1	2.3
	Equal	26	63.4	39	95.1	28	65.1	27	62.8	40	93.0	37	86.0	42	97.7	35	81.4
	Left higher	**8**	19.5	**2**	4.9	**11**	25.6	**9**	20.9	**3**	7.0	**4**	9.3	**1**	2.3	**7**	16.3

Abbreviations: See Table 1 / *Abréviations : voir tableau 1*

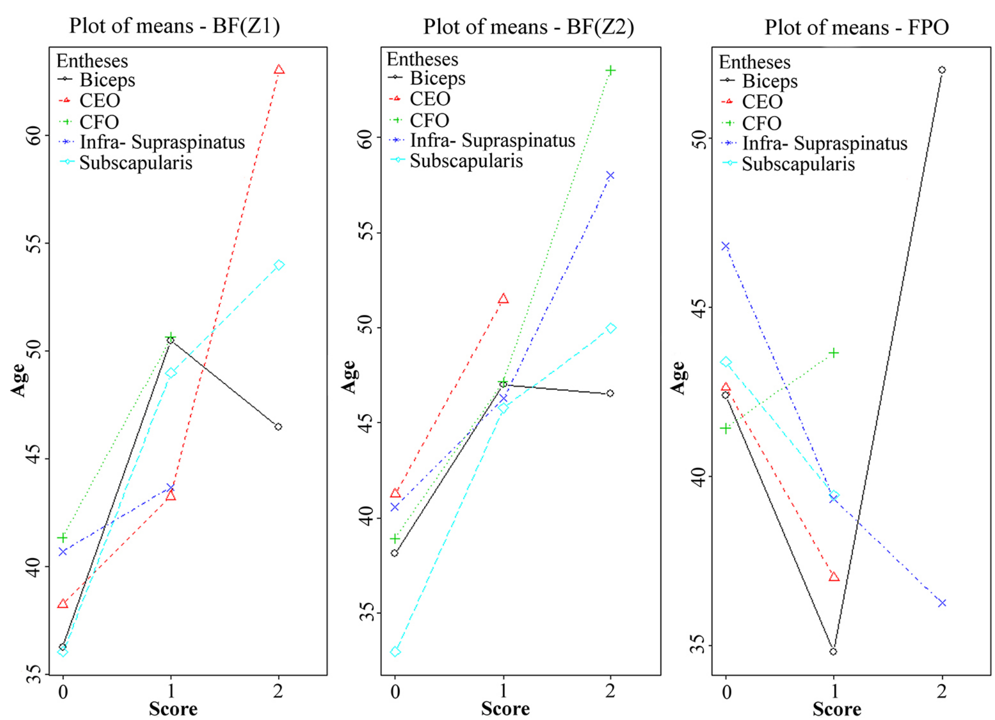

Fig. 1 Mean age by score for each enthesis showing the variability in the relationship between age and score. Abbreviations: See Table 1 / *Âge moyen par score pour chaque enthèse, illustrant la variabilité dans la relation entre âge et score. Abréviations : voir* Tableau 1

(Table 3). Fine porosity also had an association with age for two of the entheses, the left infra- and supraspinatus and the right common flexor origin, with a maximum pseudo-*R*-square of 32%. The only other statistically significant association was for erosions in zone 2 [ER(Z2)] in the left infra- and supraspinatus insertion with a pseudo-*R*-squared of only 21%. Using the whole data set, random forests support the ordinal regression by a minimal concordance between the true age and that predicted by the random forests (Fig. 2).

Discussion

Anthropological research has focussed on two aspects of EC presence: identifying occupations (activity-levels or tasks) [1–2,6–7,38] and ageing [13–17,21–22]. To determine whether it is possible to identify specific tasks or movements or even broader patterns of activity, e.g. heavy manual or nonmanual occupations, based on EC expression requires the understanding of their aetiology. From an anthropological perspective, it is easy to construct studies to take into consideration biological sex and age, and these studies have shown that this is necessary due to the increase in EC presence with age [13–17,21–22]. Previous studies of other methods have shown an increase in EC presence in fibrocartilaginous entheses with age-at-death, particularly in individuals aged over 50 [38], while others have shown an increase with age that is more pronounced for mineralised tissue forming changes, with less of an effect on osteolytic changes [22].

The test of the Coimbra method to identify the effect of age on a variety of skeletal changes in 2013 also showed that the bone formation feature in either zone was most obviously affected by age [16]. This updated study has found similar results with bone formation most consistently associated with age. Limited variability for many of the other scores made the effect of age hard to trace. It is clear that each enthesis is affected differently or has different typical expressions of ECs, making an overall pattern hard to determine. Fine porosity and textural change, in contrast to bone formation, showed higher scores in the lower ages. However, this was not completely consistent across all entheses. This is very similar to the original paper, which did not score textural change separately [16,26]. Textural change was, in the original 2013 paper, scored as part of bone formation, and this update shows the importance of distinguishing between these, as the effect of age is very different for both features [16].

This raises an important point: what is the aetiology of these features? Enthesophytes are known to be associated with traction [39,40], although others have found an association with compression [41,42]. Research also demonstrates that enthesophytes could be caused by wear and tear or they have the effect of changing loading patterns thus minimising stress-related damage [43]. Bone formation in zone 2 has also been examined histologically with woven bone as well as bone formed through endochondral ossification identified [44]. The tidemark itself, the junction between the soft non-mineralised fibrocartilage and its mineralised form, has been found to be duplicated as it is in osteoarthritic joint cartilage, indicating that similar degenerative processes are at play [45]. While this latter change cannot be identified visually in skeletal remains, it is worth bearing in mind when considering the presence of other mineralised tissue formations.

Pores and fissures have been identified histologically and some of these, those larger than one millimetre in diameter, have sometimes been found to be cysts [44]. In others blood vessels (sometimes with accompanying nerves) penetrate from the bone marrow to the edge of the mineralised fibrocartilage providing a link between the bone marrow and the enthesis such that new tissue can be formed and damage can be healed (ibid.). These are probably what anthropologists identify as porosity. In some areas of the enthesis, there is no layer of cortical bone, just mineralised fibrocartilage on top of trabecular bone (ibid.), which is likely to be identified as porosity or as an erosion in our recording system. Only elderly cadaver specimens are available to the anatomist, so finding these changes in younger individuals in skeletal remains may help to determine whether these are a response to wear and tear or are present earlier. Large fissures and macropores are highly visible on the rotator cuff entheses in adolescents during the development of the humeral head, and it is possible that some of the pores and erosions are left over from this period, but this requires further research.

Skeletal research, therefore, has a large role to play in identifying patterns of enthesis feature presence, which should improve our understanding of the causes of the changes described. What is clear from this specific piece of research is that there is a clear effect of increasing change presence with age for all changes, except fine porosity and textural change. However, it is unclear what this 'age effect' is. Ageing has numerous components and biomechanically can be considered in terms of normal age-related tissue degeneration, overuse (cumulative repetitive stress), cumulative one-off trauma or underuse injuries. The impact of these is harder to infer from skeletal remains than from *in vivo* studies. However, if normal age-related tissue degeneration were the cause, then left and right sides would be expected to show degeneration at the same rates. This study of asymmetry showed that most entheses and features had the same scores, indicating that the processes were occurring at the same rates in both sides (although it should be noted that this is also likely to be an effect of large numbers of zero scores). Where there were differences, there was a clear right side bias for all entheses, except the biceps

Table 3 Results of the ordinal regression / *Résultats de la régression ordinale*

Enthesis	Side	Feature	Estimate	Std. Error	z value	Pr(>z)	Nagelkerke pseudo R squared	2013 paper Nagelkerke pseudo R squared
Infra- and supraspinatus	Left	BFZ1	na	na	na	na	na	–
		ERZ1	na	na	na	na	na	–
		BFZ2	0.038	0.025	1.542	0.123	0.082	–
		ERZ2	0.054	0.021	2.518	0.012 *	0.205	–
		FPO	–0.038	0.018	–2.120	0.034 *	0.125	–
		MPO	0.027	0.031	0.866	0.386	0.033	–
		CA	na	na	na	na	na	–
		TC	na	na	na	na	na	–
	Right	BFZ1	na	na	na	na	na	–
		ERZ1	na	na	na	na	na	–
		BFZ2	0.027	0.031	0.866	0.386	0.033	–
		ERZ2	0.003	0.016	0.183	0.855	0.001	–
		FPO	0.001	0.016	0.088	0.930	0.000	–
		MPO	na	na	na	na	na	–
		CA	na	na	na	na	na	–
		TC	na	na	na	na	na	–
Subscapularis	Left	BFZ1	0.069	0.019	3.627	2.87E-04 ***	**0.338**	0.22
		ERZ1	0.057	0.035	1.639	0.101	0.120	na
		BFZ2	0.057	0.016	3.650	2.62E-04 ***	**0.336**	0.04
		ERZ2	0.023	0.016	1.407	0.159	0.054	na
		FPO	–0.017	0.015	–1.104	0.269	0.034	0.02
		MPO	0.030	0.028	1.078	0.281	0.043	0.04
		CA	na	na	na	na	na	na
		TC	na	na	na	na	na	–
	Right	BFZ1	0.058	0.019	3.078	0.002 **	0.261	0.21
		ERZ1	0.043	0.031	1.384	0.166	0.075	na
		BFZ2	0.076	0.019	3.999	6.37E-05 ***	0.436	0.16
		ERZ2	0.040	0.019	2.119	0.034 *	0.130	0.09
		FPO	0.022	0.016	1.348	0.178	0.050	0.08
		MPO	na	na	na	na	na	0.16
		CA	0.037	0.036	1.018	0.309	0.048	na
		TC	na	na	na	na	na	–
Common extensor origin	Left	BFZ1	0.070	0.037	1.891	0.059	0.171	0.41
		ERZ1	na	na	na	na	na	na
		BFZ2	na	na	na	na	na	0.31
		ERZ2	0.021	0.034	0.628	0.530	0.017	na
		FPO	na	na	na	na	na	na
		MPO	na	na	na	na	na	na
		CA	na	na	na	na	na	na
		TC	na	na	na	na	na	–
	Right	BFZ1	0.091	0.029	3.091	0.002 **	**0.386**	0.27
		ERZ1	na	na	na	na	na	na
		BFZ2	0.068	0.026	2.624	0.009 **	0.234	0.17
		ERZ2	0.043	0.029	1.495	0.135	0.091	0.36
		FPO	–0.029	0.034	–0.866	0.386	0.031	na

(Suite page suivante)

Table 3 *(suite)*

Enthesis	Side	Feature	Estimate	Std. Error	z value	Pr(>z)	Nagelkerke pseudo R squared	2013 paper Nagelkerke pseudo R squared
		MPO	na	na	na	na	na	na
		CA	na	na	na	na	na	na
		TC	na	na	na	na	na	–
Common flexor origin	Left	BFZ1	na	na	na	na	na	–
		ERZ1	na	na	na	na	na	–
		BFZ2	0.071	0.033	2.188	0.029 *	0.195	–
		ERZ2	na	na	na	na	na	–
		FPO	na	na	na	na	na	–
		MPO	na	na	na	na	na	–
		CA	na	na	na	na	na	–
		TC	na	na	na	na	na	–
	Right	BFZ1	0.053	0.033	1.610	0.107	0.121	–
		ERZ1	na	na	na	na	na	–
		BFZ2	0.052	0.028	1.846	0.065	0.133	–
		ERZ2	na	na	na	na	na	–
		FPO	–0.107	0.046	–2.329	0.020 *	**0.322**	–
		MPO	na	na	na	na	na	–
		CA	na	na	na	na	na	–
		TC	na	na	na	na	na	–
Biceps brachii	Left	BFZ1	0.047	0.018	2.651	0.008 **	0.190	na
		ERZ1	na	na	na	na	na	na
		BFZ2	0.038	0.018	2.161	0.031 *	0.119	0.04
		ERZ2	0.040	0.036	1.108	0.268	0.056	na
		FPO	–0.004	0.018	–0.223	0.823	0.001	0.10
		MPO	na	na	na	na	na	na
		CA	na	na	na	na	na	na
		TC	–0.033	0.019	–1.788	0.074	0.090	–
	Right	BFZ1	0.051	0.020	2.592	0.010 **	0.186	0.15
		ERZ1	na	na	na	na	na	na
		BFZ2	0.080	0.030	2.662	0.008 **	0.270	0.04
		ERZ2	na	na	na	na	na	na
		FPO	0.013	0.020	0.641	0.522	0.012	0.08
		MPO	na	na	na	na	na	na
		CA	na	na	na	na	na	na
		TC	–0.011	0.022	–0.492	0.622	0.008	–

NA indicates lack of variability, – indicates that the enthesis or feature was not tested, bold indicates a pseudo *R*-squared score > 0.29 and *indicates level of significance. The pseudo *R*-squared for this study is presented next to that from the 2013 paper [16]. Abbreviations: Table 1 / *NA indique une absence de variabilité, – indique que cette enthèse ou ce changement n'a pas été testé, les valeurs en gras indiquent un score de pseudo R carré supérieur à 0,29 et * indique une valeur* p *inférieure à 0,05. Abréviations : voir tableaux 1*

brachii insertion. A clear side difference may indicate the effects of use, but further research with a larger sample size is needed to explore which use effects are more likely to be causing the changes, due to the small variations in this study and the limited activity patterns.

Comparing the results of this paper to the original method paper shows some stark differences. Only three enthesis features demonstrated higher scores on the left side in the original paper; these were erosions at the biceps brachii insertion (both ER(Z1) and ER(Z2)) and FPO at the common extensor

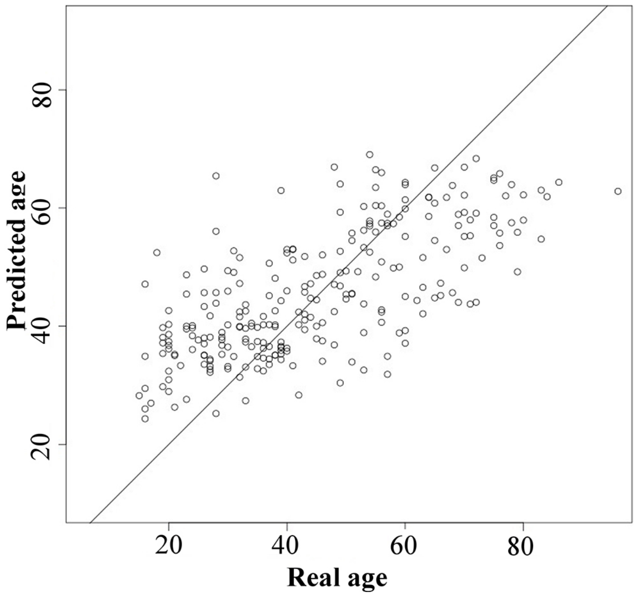

Fig. 2 True age (in years) plotted against the random forest predicted age (in years). Line indicates concordance between the true and predicted age / *Nuage de point suivant l'âge réel (en années) et l'âge prédit (en années) par les forêts aléatoires. La droite illustre une concordance parfaite*

origin [16]. While some of this may be due to a difference in the method, it is also probable that population differences and sampling effects play a role. However, biomechanical differences may also explain these results and further exploration of asymmetry is needed. In terms of the effect of age, similar patterns are seen for all features (excluding textural change which was not identified separately in the original method). The effect of age was then, as now, found to be present but was clearly not the only effect nor could the underlying cause of the effect of ageing be identified. Both studies suffer from the limited sample size available for one occupational group. Future studies are needed using larger samples with the same occupations to better characterise asymmetry and the effect of age. To study the cause of the age-related effects, large samples with multiple occupations are required, and improved methods to characterise and categorise occupations are also needed to enable identification of overuse and underuse effects.

Conclusions

The aim of this paper is to present the results of asymmetry and the effect of age on entheses and their features in a single occupation category. The study shows that feature expression varies by entheses with some features occurring only rarely at some entheses, at least in this sample. Asymmetry is rare, with predominantly higher scores on the right side where it does occur. The effect of age is minimal overall. Where there is an effect of age it tends to be towards an increased score with age, except for fine porosity and textural change, which often have a higher score in younger individuals and may indicate a developmental origin for these changes.

While there is an effect of age, it is unclear whether this is from normal age-related tissue degeneration, cumulative repetitive movement (overuse), cumulative one-off trauma or underuse injuries. Further research with larger samples of skeletal remains is needed to understand these effects. Longitudinal *in vivo* studies are also required to improve our understanding of the relationship between tendon degeneration and ECs.

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Author's contribution

All authors contributed to the research design of the study. Henderson collected the data and undertook all statistics excluding the random forest tests, which were run by Santos. All authors reviewed and critiqued the drafting, contributing to the final version. The authors have no conflicts of interest.

Conflict of interest: None.

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