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# Entheseal variation and locomotor behavior during growth

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#### 1 Abstract

2 Entheses are acknowledged as skeletal markers capable of revealing several biological and behavioral 3 aspects of past individuals and populations. However, entheseal changes (ECs) of juvenile individuals 4 have not yet been studied with a systematic approach. This contribution aims at investigating the morphological changes occurring at the femoral insertion of the gluteus maximus and tibial origin of 5 6 the *soleus* muscles to highlight a potential link between the morphological features of those entheses 7 and skeletal maturity in relation to sex, age and locomotor developmental patterns. The sample 8 consisted of 119 skeletons (age-at-death: 0-30 years) belonging to the Documented Human Skeletal 9 Collection of the Certosa Cemetery (Bologna, Italy). The entheseal variation during the last stages of 10 skeletal maturation in young adults was assessed using existing recording standards. A recording 11 protocol for each enthesis was developed for immature individuals to subdivide the morphological 12 variability into discrete categories. Univariate, bivariate and multivariate statistical analyses were 13 performed to investigate the variation of entheseal morphologies and measurements in relation to 14 bone metrics, degree of epiphyseal closure, sex, age and locomotor developmental patterns. A 15 statistically significant relationship was observed between ECs morphological patterns and age for 16 both entheses, while sexual differences were negligible. A relationship between ECs morphological 17 pattern and locomotor milestones emerged only for the gluteus maximus. Even though further testing 18 is needed on other documented skeletal collections, our protocol could be usefully applied in forensic 19 and archaeological fields and serving as important reference for evolutionary investigations.

20

#### 21 Keywords

22 Entheseal Changes; Locomotor Development; Documented Skeletal Collections; Skeletal Maturation

23

#### 24 **1. Introduction**

25 The entheses are the areas where tendons, ligaments and joint capsules attach to the bone (Benjamin 26 et al., 2002), and represent the only direct evidence of the musculotendinous system on skeletal 27 remains. The entheseal changes (ECs; this term designates all alterations of entheses seen in the 28 skeletal material; Jurmain and Villotte, 2010) have been largely explored in the last decades. The 29 entheses are physiologically subjected to significant mechanical stress, which inevitably leads to 30 some reaction in the bone tissue. Even if the extent to which the mechanical stress influences 31 entheseal morphology with respect to other factors (age, hormonal factors, etc) cannot be ascertained, 32 entheses are widely uses in the attempt to reconstruct biological and behavioral aspects (Dutour, 1986; 33 Hawkey and Merbs, 1995; Kennedy, 1998; Weiss, 2003; Belcastro et al., 2006; Mariotti and 34 Belcastro, 2011; Villotte and Knüsel, 2013; Belcastro and Mariotti, 2017; Belcastro et al., 2020;

Karakostis and Harvati, 2021; Karakostis et al., 2021). However, ECs expression (robusticity and 35 36 pathological features) has a multifactorial etiology, where the aging process in adulthood is one of 37 the main factors involved (Cunha and Umbelino, 1995; Robb, 1998; Mariotti et al., 2004, 2007, 2009; 38 Villotte, 2009; Alves Cardoso and Henderson, 2010; Villotte, 2009; Villotte et al., 2010; Niinimäki, 39 2011; Milella et al., 2012; Villotte and Knüsel, 2013). As far as sex is concerned, some dimorphism 40 has been observed in adults, often, but not always, corresponding to a greater entheseal robusticity in 41 males (Mariotti et al., 2007; Alves Cardoso and Henderson, 2010; Milella et al., 2012). Different 42 observational recording standards have been so far developed, but only on bones of adult individuals 43 (e.g., Hawkey and Merbs, 1995; Mariotti et al., 2004, 2007; the Coimbra method by Henderson et al., 44 2016, 2017), while little attention has been given to the ECs during growth in juvenile skeletons.

45 Our previous investigations have shown different patterns in some lower limb entheseal 46 morphologies between Neanderthals (Krapina, 130 000 BP and El Sidrón, 49 000 BP) and modern 47 humans (Belcastro et al., 2006; Mariotti and Belcastro, 2011; Belcastro and Mariotti, 2017; Belcastro 48 et al., 2020). Our results showed that the morphological variability of the gluteus maximus enthesis 49 exceeded that observed in modern humans, while the variability of the soleus was comparable to 50 modern humans'. In detail, we observed a low intrapopulation variability in each Neanderthal sample 51 (despite small sample size) between the adult and juvenile morphology of gluteus maximus muscle, 52 and a large intrapopulation variability in modern humans between the adults and juvenile individuals 53 on the same enthesis. Furthermore, we empirically observed that modern juveniles exhibited 54 characteristics that exceeded the variability seen in the adults and the absence of standardized 55 recording systems hindered our ability to quantify these differences. Many entheses in the adults were 56 completely covered by variably developed *mineralized tissue formations* (Villotte et al., 2016), 57 formerly known as crests or ridges (Peterson, 1998; Robb, 1998; Hawkey and Merbs, 1995; Eshed et 58 al., 2004; Mariotti et al., 2007; Milella et al., 2012). Juvenile individuals, on the other hand, 59 systematically presented surface discontinuities (like diffuse porosity and furrows) covering the entire 60 entheseal surface, as also previously observed by other authors (Matyas et al., 1990; Wei and 61 Messner, 1996). A recording standard for juvenile entheses has been recently published by Palmer et 62 al. (2023). However, of age (Bly, 1994)the authors just score a set of features already known in the 63 literature (e.g., enthesophytes; cf. Villotte et al., 2016) that could be recorded on any individuals 64 regardless to their age class. On the contrary, in the present work, we specifically investigate the 65 variability of juvenile entheseal morphologysex and , focusing on surface architecture and texture. 66 These features allow to univocally recognize juvenile morphologies. Additionally, we had the 67 possibility to study a much larger sample where most of the age classes are better represented.

68 Our work is aimed at investigating the entheseal morphometric variability of the femoral 69 insertion of gluteus maximus (hereinafter "GM") and tibial origin of soleus (hereinafter "SOL") 70 muscles (already studied in the Neanderthal samples) in juvenile individuals in relation to age, sex 71 and locomotor pattern during growth, adding new insights on the biological and biomechanical 72 aspects of bipedalism, from an ontogenetic and evolutionary point of view, already explored in our 73 research group also with other approaches (Sorrentino et al., 2020a, 2020b, 2020c, Figus et al., 2022, 74 2023; Pietrobelli et al., 2022a, 2022b, 2023; Colombo et al., 2019). In detail, we developed a 75 recording standard method to better classify the entheseal (continuous) variability into discrete classes 76 and verify its applicability in response to the locomotor pattern (detailed below). In this frame, the 77 availability to access to the Documented Human Skeletal Collection (Belcastro et al., 2017, 2022), 78 sampling individuals with known sex and age, allowed us for trying to meet those objectives.

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# 80 1.1. GM and SOL in locomotion development

GM and SOL muscles are highly involved in human bipedal locomotion. The human gait cycle can be divided into two phases (Whittle, 2006; Neumann, 2009): a stance phase, where the foot is in contact with the ground, and a swing phase, where the foot swings before touching the ground. The GM and the SOL are both involved in the stance phase. Specifically, the GM is involved from the moment the heel contacts the ground (i.e., Initial Contact) and remains active until the moment the heel leaves the ground (i.e., Heel Rise) to promote hip extension; the SOL, instead, contracts during late mid-stance and terminal stance to promote plantar flexion and control dorsiflexion.

88 During the first six months after birth the infant performs precursory locomotor movements 89 like supine kicking and supported sitting, while weight-bearing on lower limbs is completely absent 90 (Thelen and Fisher, 1982; Thelen et al., 1984). Afterwards, the child goes through a short period (up 91 to 8 months) of dependent/independent crawling and scooting. Infants typically gain a standing 92 position and start cruising towards the end of the first year of life, at first while holding on to objects 93 or caregivers for support and eventually transitioning to independent toddling (Bly, 1994; Adolph et 94 al., 1998). From about one year of life, the toddler goes through various stages of maturation of their 95 locomotor behavior and towards the age of 6 they acquire the mature bipedal gait typical of adults. In 96 the early phases of toddling (i.e., between the ages of 1 and 2 years), children lean on a wide base of 97 support, abducting their thigh and flexing their hip and knee. They point toes outwards and strike the 98 ground with a plantigrade foot (McGraw, 1940; Forssberg, 1985; Hallemans et al., 2003; Hallemans 99 et al., 2006a, b). As a result of a flexed hip and knee, the torso tends to lean forward causing the hip 100 (contralateral to the standing leg) to lift during the swing phase and the pelvis to tilt from side to side 101 (Hallemans et al., 2004; Cowgill et al., 2010). This early form of walking is typically conducted at a 102 slow pace, with small and jerky steps performed in bursts at irregular intervals (Hallemans et al., 103 2006a). Children at age 3 usually engage in a more mature toddling pattern, with improved gait, 104 narrower and longer steps, and a loading pattern of an initial heel-strike which sees the beginning of 105 the stride with the center of pressure under the calcaneus (Adolph et al., 2003; Ivanenko et al., 2004; 106 Hallemans et al., 2006b; Zeininger et al., 2018; Swan et al., 2020). Finally, around age 6 (on average), 107 a mature, stable, and efficient gait is fully acquired, as a result of the progressive increase in the femoral bicondylar angle (that adducts the knee, positioning the joint under the body's center of 108 109 gravity) which leads to the correction of the genu varum typical of toddlers (Tardieu and Trinkaus, 110 1994; Swan et al., 2020).

111 From the locomotor behavior illustrated above, during growth, it can be deduced that the GM 112 and SOL muscles begin to play an important role towards the end of the first year of life, i.e., when 113 the infant begins to be able to stand and take their first steps independently. GM and SOL control 114 respectively hip flexion and dorsiflexion during standing. The GM is certainly involved even within 115 the first year for precursory locomotor movements such as supine kicking, dependent/independent 116 crawling and scooting, or even during phases of assisted locomotion while holding on to objects or 117 caregivers (Thelen and Fisher, 1982; Thelen et al., 1984; Bly, 1994; Adolph et al., 1998). On the 118 contrary, we are not aware that the SOL (with the plantar flexion movement) plays any relevant role 119 among the precursory locomotor movements. However, it is reasonable to think that GM and SOL 120 begin to make a significant contribution only when external supports for locomotion disappear, and 121 even more so when a mature and efficient bipedal locomotion is acquired around the age of 6.

Since all the entheses respond directly to the biomechanical stimuli imparted by muscle activity we believe it is very important to take the locomotor development into account as a potential etiological agent of any entheseal modifications in subadults.

125

#### 126 **2. Materials and Methods**

In this work we examine the morphometric variability of the femoral insertion of the *gluteus maximus*muscle (GM) and tibial origin of the *soleus* muscle (SOL). Both entheses are fibrous (Havelková and
Villotte, 2007; Villotte, 2009; Mann and Hunt, 2012, p. 204; Weiss, 2015; Milella et al., 2020; Villotte
and Santos, 2022. entheses).

The sample consists of 119 skeletons of juvenile individuals aged from birth to 30 years (Table 1) belonging to the Documented Human Skeletal Collection of the "Certosa" Cemetry of Bologna (Italy), for which the personal data are known from the cemeterial records (Belcastro et al., 2017, subsample of young adults was included in order to observe the morphological variation of the entheses with respect to the last stages of skeletal maturation. Only well-preserved individuals were included in this research, discarding all those where it was impossible to evaluate at least one enthesis on a femur or tibia (i.e., due to taphonomic alterations). Furthermore, the enthesis was considered recordable if at least 50% of its surface was not damaged. Individuals whose femurs or tibiae showed evidence of pathologic conditions (e.g., presence of abundant woven bone, deformations of the diaphysis) were also discarded. The individuals analyzed in this study were divided into 7 age classes (Table 1), where the first two age classes were designated considering the information present in the literature about the early stages of acquiring bipedal gait:

Age class 1 (<1 years): bipedal locomotion is absent (Thelen and Fisher, 1982; Thelen et al.,</li>
144 1984; Bly, 1994; Adolph et al., 1998).

• Age class 2 (1-5.9 years): bipedal locomotion is present but still immature (toddling). This is therefore a long transitory phase that goes from a rudimental and still dependent locomotion to a mature gait (McGraw, 1940; Forssberg, 1985; Adolph et al., 2003; Hallemans et al., 2003, 2004; Ivanenko et al., 2004; Hallemans et al., 2006a, b; Cowgill et al., 2010; Zeininger et al., 2018; Swan et al., 2020; Pietrobelli et al., 2022a).

• Age class 3 (6-10.9 years), 4 (11-15.9 years), 5 (16-20.9 years), 6 (21-25.9 years), 7 (26-30 years): even though from the age of 6 a bipedal gait is already completely acquired, the remaining individuals have also been divided into quinquennial classes, (except for the last one which covers 4 years) to identify any possible morphological changes in entheses in relation to bone lengthening (classes 3-5) and entheseal "settlement" once the definitive stature has been reached (classes 6-7).

We examined juvenile femurs and tibiae without considering the age a priori. The variation 155 156 observed at the GM was subdivided into 3 morphological classes, whereas variation at the SOL was 157 subdivided into 4 morphological classes, as described in Table 2 and depicted in Figures 1-7. At each 158 morphological class we arbitrarily assigned a number that is not in a predefined order. The assessment 159 of ECs on GM and SOL in juvenile individuals was therefore performed following a new descriptive 160 and photographic standard we created for this purpose (Table 2; Figures 1-7). For each morphological 161 class, photographs of four entheses belonging to four different individuals were provided to better 162 illustrate the variability within the single morphological classes (Figures 1-7). The entheses which 163 were completely covered by mineralized tissue formations, in particular characterized by diffuse 164 cortical irregularities and longitudinal protrusions (typical features of adult individuals) have been 165 assessed with Mariotti et al. (2007) method for entheseal robusticity.

We proceeded taking the linear dimensions of the entheses with a digital sliding caliper (resolution: 0.001 mm):

• The **entheseal length** was taken by measuring the distance between the most proximal and most distal extremities, following the longitudinal axis of the enthesis, regardless of its relative orientation to the bone length. In the case of GM, a possible third trochanter must be included in the measurement, as part of the enthesis. Both extremities must be clearly visible, otherwise the measurement must be considered non-recordable.

• The **entheseal width** was measured at maximum width, therefore according to the transversal axis of the enthesis. Regarding the GM, it is essential that the proximal half of the enthesis is intact, as in this area the gluteal tuberosity is very often wider and more evident. If not, the width must be considered non-recordable. Concerning the SOL, at least the 50% of the enthesis must be intact, regardless of whether it is proximal or distal, as the enthesis does not appear to have significant variations in width along its attachment.

179 The stage of development of femurs and tibiae was assessed by determining of the degree of 180 epiphyseal closure and bone size. The degree of epiphyseal closure was assessed following the Belcastro et al. (2019) method, which provides a five-degree assessment standard. For statistical 181 182 convenience, the different degrees of closure of the epiphyses evaluated with this method were 183 reduced to three: 0 corresponds to not fused (equivalent to grade 0), 1 corresponds to partially fused 184 (equivalent to grades 1 and 2) and 2 corresponds to totally merged (equivalent to grades 3 and 4). 185 This adaptation was then extended to the whole bones to assign an overall assessment of its state of 186 maturation: in grade 0 none of the epiphyses are fused, in grade 1 at least one epiphysis is at closure 187 stage 1 or 2, and in grade 2 all bone epiphyses must be fully fused (grade 3 and 4).

Linear measurements of maximum length and transverse diameter at midshaft were taken following the protocols provided by Martin and Saller (1957). Immature bones were instead measured according to Fazekas and Kósa's (1978) protocol. The measurements were considered non-recordable if the cortical bone at the landmarks was damaged.

All statistical analyses were performed in R v.4.2.2. To test the validity of this method to morphometrically assess the GM and SOL entheses, intra- and inter-observer errors were evaluated calculating the Cohen kappa coefficient ( $\kappa$  – Cohen, 1960, 1968) and the accuracy for qualitative variables (i.e., the morphological standards), while the intraclass correlation coefficient (ICC – Fisher, 1954) was opted for the quantitative variables (i.e., all the linear entheseal measurements). The author who executed the inter-observer error had no previous experience on the study of ECs of GM and SOL in juvenile individuals.

199 Chi-squared tests  $(X^2)$  (Pearson, 1900) were performed to test differences in sex and age 200 distribution within the sample and a t-test (Student, 1908) was calculated to evaluate a possible 201 asymmetry between the left and the right side. Descriptive statistics (mean, standard deviation, 202 median, minimum and maximum values) were calculated for each measurement and grouped by age 203 class and sex. The distribution of the morphological classes of GM and SOL by age and sex was 204 represented through boxplots. Normality distribution was assessed with a Shapiro-Wilk normality 205 test (Shapiro and Wilk, 1965). Fisher's exact tests of independence (Fisher, 1934) were calculated to 206 study the distribution of the morphological classes of entheses by sex, by age classes and by sex 207 within the single age classes. Wilcoxon rank-sum tests (Wilcoxon, 1945) were performed to analyze 208 the linear measurements in relation to sex within the age classes. The data were also investigated for 209 possible correlations performing Spearman's tests (Spearman, 1904) between the morphological 210 classes of the entheses and age, linear measurements and epiphyseal closure degree; linear regression 211 models have also been developed for these same variables. The data were finally analyzed performing 212 a Factor Analysis of Mixed Data (i.e., FAMD) using the "FactoMineR" (Lê et al., 2008) and 213 "factoextra" (Kassambara and Mundt, 2020) packages. Specifically, the following variables were 214 included in the FAMD: sex, age, age classes, entheseal morphological classes, linear measurements 215 of the bones and entheses and degree of epiphyseal closure. For this purpose, missing data were 216 replaced with each variable's median value for linear measurements, calculated for each age class 217 and sex.

218

#### 219 **3. Results**

No significant differences were found in the distribution of the sexes by age ( $X^2 = 59$ ; *p*-value >0.05), but differences were detected in the distribution of individuals among the age classes ( $X^2 = 82.6$ ; *p*-value <0.0001). In fact, age class 1 is largely the most represented (especially by males), while age classes 3, 4 and 7 are the least represented. In age class 4 the females are totally absent (Table 1). No differences were found between left and right limb, therefore all the following statistical analyzes were performed only on the left limb. Since not all variables followed a normal distribution or were homoscedastic, nonparametric tests were chosen for the univariate and bivariate statistical analyzes.

The results of the intra- and inter-observer errors are shown in Table 3. The intra-observer results show a high reliability, both concerning the morphological standards and the linear entheseal measurements. The inter-observer results, instead, show very reliable results concerning SOL\_morph and all the linear entheseal measurements of GM, while a poorer but still fair/moderate reliability (Landis and Koch, 1977; Koo and Li, 2016) resulted regarding GM\_morph, SOL\_length and SOL\_width.

The most represented morphological classes of GM are GM1, GM2a and GM2b, with a way larger male contribution in class GM2a; regarding the SOL, the most represented morphological class is SOL3 (Table 4). For both GM and SOL, statistically significant differences were only found among age classes. Within age classes significant differences between sexes emerged only in GM in age class 1 (Table 4). Figure 8 shows for both sexes that as the age increases, morphological classes GM2a, GM2b, GM1 and GM3 follow one another almost without overlapping. A similar pattern can
be seen for SOL, where SOL1+SOL2, SOL3 and SOL4 seem to follow one another, even though
there does not appear to be a clear separation between SOL1 and SOL2.

241 Table 5 and Table S1 (i.e., the extended version of Table 5) show the descriptive statistics for 242 linear measurements of bones and entheses by sex and age classes and the results of the Wilcoxon 243 rank-sum test performed by sex within the age classes for each continuous variable; the results of the age classes 3, 4 and 7 and the result of the entheseal length of SOL (SOL\_length) in class 1 were not 244 245 reported due to the small number of observations. By and large females show comparable 246 measurements to males during growth, except for the bone diameters in age class 1 247 (Femoral\_diameter and Tibial\_diameter). A more marked sexual dimorphism begins to appear from 248 age class 5. In general, all measurements tend to increase with age.

249 Table 6 shows the results of the Spearman correlation and linear regression performed 250 between the entheseal morphological classes and age and other variables inherently correlated with 251 age (linear measurements of bones and entheses and epiphyseal closure), both with the distinction 252 between the sexes and together. To perform correlation and linear regressions, it was necessary to 253 convert the morphological classes into ranks. Since the descriptive statistics relating to the 254 morphological classes of GM, more specifically in Figure 8, showed a clear sequence in relation to 255 the age of morphologies GM2a, GM2b, GM1, GM3 and Mariotti subclasses, it was preferred to assign 256 the ranks consistently with this pattern. Regarding the morphological classes of SOL, the rank 257 conversion was performed following the original sequence (SOL1, SOL2, SOL3, SOL4, Mariotti 258 subclasses), as it did not show big discrepancies compared to the pattern shown in the Figure 8. In all 259 the cases, all the considered variables resulted strongly and positively correlated with the 260 morphological classes assigned to the two entheses. Most of the linear regressions show a good predictive power ( $r^2 > 0.5$ ), except for all the linear measurements of tibia and SOL. 261

262 Two FAMDs were calculated, one for the GM and all the variables regarding the femur and 263 one for SOL and all the variables regarding the tibia (Figures 9 and S1). In the FAMD calculated for 264 GM morphological classes and related variables, Dim1 explains the 34% of the variance of the 265 dataset, while Dim2 explains the 9% (Figure 9a, b; Figures S1a and S2a). In the FAMD for SOL 266 morphological classes and related variables, instead, Dim1 explains the 37.5% of the variance of the 267 dataset, while Dim2 explains the 9.2% (Figure 9c, d; Figures S1b and S2d). In both cases, Dim1 is 268 mostly driven by age classes, age, all linear measurements, degree of epiphyseal closure and 269 morphological classes (Figure S2b, e); age classes, morphological classes and the degree of 270 epiphyseal closure highly contribute to Dim 2 too (Figure S2c, f). In both cases sex contributed very 271 little (Figures S1a, b and S2b, c, e, f). As far as the GM and femoral variables are concerned (Figures

272 9a, b and S1a), Dim 1 clearly separates GM2 and GM3+Mariotti subclasses while GM1 encompasses 273 almost all the variability along Dim1, but only considering its confidence interval. A pattern is thus 274 visible (especially by observing the points), which sees a succession among the morphological classes 275 GM2a, GM2b, GM1 and GM3. Dim2 explains the separation between GM1+GM2 and Mariotti 276 subclasses, while GM3 overlaps with Mariotti for higher values and with GM1+GM2 for lower values 277 of Dim2 (Figure 9a). The age classes follow the same pattern, where age classes 1, 2, 3, 4 and 5 plot 278 close to morphologies GM2a, GM2b and GM1, while age classes 6 and 7 are closer to GM3 and the 279 Mariotti subclasses (Figure 9a). Figure 9b shows a complete separation along Dim1 between the 280 individuals who present unfused femoral epiphyses and who present a partial or total state of closure. 281 Morphological classes GM2a and GM2b are strongly associated to unfused femoral epiphyses, GM1 282 is associated to both unfused and partially/totally fused epiphyses, GM3 is associated to both partially 283 and totally fused epiphyses, and Mariotti subclasses are all associated to totally fused epiphyses. 284 Regarding the SOL and tibial variables (Figure 9c, d; S1b), the morphological classes SOL1+SOL2, 285 SOL3 and SOL4 separate from one another along Dim1, while the separation among SOL4 and the 286 Mariotti subclasses is better explained by Dim 2, although they are still largely overlapped, especially 287 in the negative values of Dim2 (Figure 9d). The confidence intervals of SOL1 and SOL2 do not 288 separate neither along Dim1 nor along Dim2, resulting largely overlapped (Figure 9c); furthermore, 289 SOL1 and SOL2 morphologies fall entirely within the confidence interval of SOL3 (for more negative 290 values), however, the overlap between the points is minimal. The age classes follow the same pattern, 291 where age classes 1, 2, and 5 plot closer to morphologies SOL1, SOL2, SOL3 and SOL4, while age 292 classes 6 and 7 result closer to the Mariotti subclasses (Figure 9c). Figure 9d shows a complete 293 separation along Dim1 between the individuals who present unfused tibial epiphyses and who present 294 a partial or total state of closure. Morphological classes SOL1, SOL2 and SOL3 are strongly 295 associated to unfused femoral epiphyses, while class SOL4 and Mariotti subclasses are associated to 296 partially and totally fused epiphyses. The packages FactoMineR and factoextra excluded the age 297 classes 3 and 4 from the calculation of this FAMD because some variables were not represented in 298 these age classes (i.e., SOL\_length in both age classes 3 and 4, SOL\_width as regards the females of 299 age class 4).

300

#### 301 4. Discussion

302 Our work sheds light on the variability of ECs during growth highlighting the influence of age, sex303 and locomotor development.

The results concerning the calculation of the errors (Table 3) showed an overall lower agreement on the inter-observer error, especially on the morphological standard of GM (GM\_morph), 306 entheseal length of SOL (SOL length) and width (SOL width). As far as concerns GM morph, the 307 disagreement was almost entirely caused by a difficulty in recognizing the GM1 morphology, specifically, the second observer tended to recognize as GM3 the entheses that the first observer, 308 309 creator of the standard, recognized as GM1. The discrepancy observed for SOL length and 310 SOL\_width is likely due to the fact that this enthesis is often discontinuous and not well-defined, 311 especially in its proximal extremity. The potential difficulty in identifying clear boundaries in 312 entheses has already been raised in the past (Zumwalt, 2005), however, it was not an obstacle to the 313 research as the most uncertain measures were removed from the analysis.

The morphological variability observed on entheses is continuous, it is self-evident that it is very difficult to divide it into discrete categories and therefore to grasp recurring characteristics. fssStudies on enthesis are generally affected by the experience of the observer (Wilczak et al., 2017),however, the protocol here proposed has the advantage of being easily usable, with acceptable levels of repeatability, and of allowing the evaluation of numerous samples without the need of expensive equipment.

320 Remarkable sex differences emerged neither in the univariate nor in the multivariate statistics 321 (Table 4; Figure S1a, b). The distribution of morphological classes of both GM and SOL do not differ 322 between the sexes, except for a slightly significant difference within age class 1 in GM (Table 4), 323 probably related to the imbalance of the *sex ratio* present in this age class: firstly, because there are 324 way more males than females (Table 1), secondly, because the youngest individuals of age class 1 325 are male, while the oldest are mostly female (10 females vs 25 males in the first 6 months, and 10 326 females vs 3 males in the last 6 months). It is likely that this imbalance is also responsible for the 327 significance that emerged in the same age class as regards the transverse diameter at midshaft of the 328 femur (Femoral\_diameter) and tibia (Tibial\_diameter), which sees higher dimensions in females 329 rather than males (Tables 5 and S1). Apart from the differences just mentioned, we did not observe 330 significant sex differences in measurements in the first three age classes (i.e., from birth to 10.9 years 331 of age), contrary to what reported in literature for other metric variables (e.g., Malina and Johnston, 332 1967; Humphrey, 1998; Stull and Godde, 2012; Stull et al., 2017; Luna et al., 2017; Marino et al., 333 2020). The present study also disagrees with Gonen Aydin et al. (2021), who observed differences 334 between sexes in the gait cycle pattern.

Fisher's exact test of independence (Table 4), Spearman correlation tests and linear regressions (Table 6) and the FAMDs (Figure 9 and S2) reveal the significant role of **age** in explaining variation among the morphological classes of both GM and SOL. Indeed, several studies conducted on adults highlighted a strong relationship between entheseal morphological features and age (Hawkey and Merbs, 1995; Peterson, 1998; Robb, 1998; Eshed et al., 2004; Mariotti et al., 2007;

Alves Cardoso and Henderson, 2010; Milella et al., 2012). This is immediately evident in the 340 341 descriptive statistics (Figure 8), where the different morphologies follow one another in order of age 342 and characterize well-defined age intervals, especially as regards the GM morphological classes. This 343 pattern and the close relationship with age tends to be less clear considering the Mariotti subclasses 344 (we remind that Mariotti and colleagues' assessment method is applicable on adult individuals only, 345 in this case on long bones with fully closed epiphyses). The FAMD divided by morphological class 346 of the GM (Figure 9a) shows a pattern that sees a distancing between the new morphological classes 347 (i.e., GM2a, GM2b, GM1 and GM3) along Dim1, where age and related variables play a predominant 348 role (Figure S2b), despite a partial overlapping of the confidence intervals. A similar pattern can be 349 seen in the FAMD divided by the morphological classes of the SOL (Figure 9c): the new 350 morphological classes (i.e., SOL1, SOL2, SOL3 and SOL4) result divided along Dim1, with the 351 exception, however, of classes SOL1 and SOL2, whose centroids are very close to each other. On the 352 other hand, for both GM and SOL, the variability of individuals associated with the Mariotti 353 subclasses is best explained by Dim2, where the linear dimensions of bones and entheses play a 354 secondary role (Figure S2c, f), as growth has ceased. Furthermore, the confidence intervals of the 355 single Mariotti subclasses do not separate considerably: this result is not surprising, as there were 356 very few individuals with an enthesis characterized by a complete mineralized tissue formation (and 357 therefore detectable with the method developed on adult samples by Mariotti et al., 2007), moreover, 358 5 morphological subclasses were considered, which are a lot compared to the few individuals.

359 As regards the relationships between entheseal morphology and **locomotor** milestones, we 360 here consider the EC patterns observable within the first two age classes, as the child acquires a 361 mature locomotor behavior by the age of six (i.e., by the end of age class 2) with the correction of the 362 genu varum (Tardieu and Trinkaus, 1994; Swan et al., 2020). For the GM, the GM2a morphology 363 seems to uniquely characterize age class 1, while morphology GM2b characterizes age classes 1 and 2, but with a greater frequency in age class 2. This morphological switch may reflect a locomotor 364 365 pattern, in fact, just at the end of the first year of life (i.e., age class 1) the infant begins to take their 366 first steps independently (Bly, 1994); therefore, we hypothesize that this new stimulus may represent 367 the cause of this morphological change. Until the age of six, toddlers present a poorly accentuated 368 bicondylar angle of the femur, which causes poor medio-lateral control during locomotion (Tardieu 369 and Trinkaus, 1994; Swan et al., 2020). This skeletal feature causes a "waddling" gait (Cowgill et al., 370 2010) which we suppose could affect the GM entheseal morphology in this phase. The hypothesis 371 that morphology GM2b may be determined by an immature locomotor behavior is supported by the 372 fact that from age class 3, when the child has fully acquired a complete bipedal locomotion, 373 morphology GM2b disappears, leaving room for morphology GM1 only, characterized by a fine porosity and a much smoother surface. The overlap between GM2a and GM2b in class 1 and between
GM2b and GM1 can reflect variability among children in the maturation of their locomotor skills
(Bly, 1994). The close relationship between gait biomechanics and age here proposed is also
supported by Froehle et al. (2013) and Liu et al. (2022).

378 The relationship between the morphology of the entheses and locomotor milestones is not 379 clear for SOL instead: age class 1 is characterized by morphology SOL1, SOL2, but above all by 380 SOL3, which is present up to age class 5 (Table 4). Probably these morphologies are not associated 381 to a particular locomotor pattern. One reason that can explain this big difference between the two 382 entheses is that the stimulus imparted from the two entheses on the bones can differ for several 383 reasons. Firstly, the SOL enthesis is an origin, moreover, shared with another bone (i.e., the fibula); 384 on the other hand, the GM enthesis is an insertion, which consequently has to bear a much greater 385 effort since it is located on the bone that performs the movement. Firstly, the SOL enthesis serves as 386 an origin (with another end originating from the fibula). In contrast, the GM enthesis functions as an 387 insertion and is therefore subject to a significantly greater effort due to its location on the bone 388 responsible for the movement. This anatomo-functional difference could also explain why the 389 insertion of the GM on the femur is always visible and much more defined than the tibial origin of 390 the SOL. Moreover, the GM muscle seems to activate before the SOL muscle during life, in fact, it 391 seems to be already involved in the precursory locomotor movements, while the SOL does not seem 392 to have any noteworthy role before the acquisition of an upright posture (Bly, 1994).

393 Regarding the relationships between entheseal morphology and **epiphyseal closure**, in both 394 the entheses here analyzed, a single morphology dominates in age class 3 (6-10.9 years of age), which 395 are morphological classes GM1 and SOL3 (Table 4). In the following phases, the entheses seem then 396 to evolve into forms characterized by a mineralized tissue formation typical of adults (Villotte et al., 397 2016). In both entheses, however, this shift from a "juvenile-like" to an "adult-like" morphology does 398 not seem to be sudden, in fact, it goes through a sort of "transitional" phase in which a coexistence of 399 typically adult (raised areas) and juvenile (porotic or furrowed zones) characteristics is observed: 400 these are respectively morphology GM3 (Table 4; Figures 3, 8 and 9a) and morphology SOL4 (Table 401 4; Figures 7, 8 and 9c). This relevant morphological change, or rather at this point, this maturation 402 process of the enthesis, could be triggered by the closure of the epiphyses, that marks the end of the 403 long bone lengthening and thus the "migration" of the enthesis along the diaphysis (Hoyte and Enlow, 404 1966; Dörfl, 1980a, 1980b; Hurov, 1986). This hypothesis may be supported by the results of the 405 FAMD divided by the degree of epiphyseal closure (Figures 9b, d). In Figure 9b, relating to the 406 enthesis of the GM, morphology GM3 and the Mariotti subclasses (typical of adults) are strongly 407 associated with individuals who present partially or totally fused epiphyses. GM1 instead appears to

be associated with all closure degrees, but more commonly with unfused and partially fused epiphyses, while morphological classes GM2a and GM2b are exclusively associated with unfused epiphyses. Given the observed pattern, considering the close correlation between morphologies and age (Table 6) and the division between morphologies observed in Figure 9a, it can be hypothesized that morphology GM1 remains for a short period when the epiphyses begin to fuse, after which a process of gradual mineralization of the enthesis begins and finally leads to a complete ridge.

414 The enthesis of SOL shows the same pattern (Figure 9d): the "transitional" morphology SOL4 and 415 the Mariotti subclasses for SOL (typical of adults) are strongly associated with individuals who 416 present at least partially fused tibial epiphyses, in which, therefore, bone lengthening has already 417 ceased. Morphological class SOL3, although associated much more with unfused epiphyses, is also 418 present in several individuals with partially fused ones, while morphological classes SOL1 and SOL2 419 are only associated with unfused epiphyses. The same conclusions drawn for the GM can reasonably 420 be applied on the SOL as well, so considering the close correlation between morphologies and age 421 (Table 6) and the division between morphologies observed in Figure 9c, it can be hypothesized that 422 morphology SOL3 remains for a short period when the epiphyses begin to fuse, after which a process 423 of gradual mineralization of the enthesis begins, finally leading to a complete ridge.

These transformations observed in GM and SOL entheses are attested by the possible presence of woven bone in the "transitional" morphologies GM3 and SOL4 (Table 2, Figures 3 and 7), which may be an indicator of ongoing mineralization (White et al., 2012; Cunningham et al., 2016, pp: 26– 29, 34–35).

To sum up, we saw a transformation from a very irregular appearance typical of subadults to a *mineralized tissue formation* in variable degrees of expression (Mariotti et al., 2007). The porotic or furrowed surface of juvenile entheses could therefore be symptoms of a strong bone remodeling due to the migratory process, as Hoyte and Enlow (1966), Dörfl (1980a, 1980b) and Hurov (1986) proposed, which prevents the mineralization of the bone-tendon interface.

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# 434 **5.** Conclusions

The present work sheds light on aspects never explored before, providing new important insights into the variability of ECs in modern juvenile individuals. In particular, we gathered more consistent data than before (cf. Belcastro et al., 2020) on the modern juvenile morphological variability. It would be interesting to enlarge the small sample of Neanderthal adult and juvenile entheses to perform more consistent comparisons between the intrapopulation variability of these different human populations. Through the application of our morphometric recording standard for modern juvenile

individuals, we highlight a very clear relationship between the morphometric changes affecting the

entheses of GM and SOL muscles and age. For the GM, also a relationship between the morphological
ECs and locomotor milestones seems to be observed. Another interesting perspective of this study is
the possibility of using these features in subadult-young adult age estimation, with possible
applications in bioarcheological and forensic fields. In fact, these methods would permit to estimate
the age of juvenile individuals, even from fragmented bones, provided that the enthesis is preserved.

447 A future goal will certainly be to test our protocol on other documented juvenile skeletal 448 collections, especially enlarging the sample in less represented age classes in this study, in order to 449 deepen the understanding of the relationship between entheseal morphometric changes and 450 development, also in an evolutive perspective.

451

### 452 Author contributions

453 Davide Mameli: conceptualization; methodology; validation; formal analysis; investigation; writing
454 - original draft; writing - review and editing. Annalisa Pietrobelli: conceptualization; validation;
455 formal analysis; writing - review and editing. Rita Sorrentino: writing - review and editing. Teresa
456 Nicolosi: writing - review and editing. Valentina Mariotti: conceptualization; supervision; writing 457 review and editing. Maria Giovanna Belcastro: conceptualization; resources; supervision; project
458 administration; writing - review and editing.

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## 763 Supporting information

**Table S1**. Descriptive statistical analysis (mean, standard deviation, median and min-max values) for
linear measurements of the femur and GM enthesis (left) and tibia and SOL enthesis (right) with the
distinction between sex and age class (*Extended version of Table 5, main text*). All values are
expressed in mm.

## 768 (*The table should be placed here*)

- Note. Age class 1: <1 year; age class 2: 1-5.9 years; age class 3: 6-10.9 years; age class 4: 11-15.9
- years; age class 5: 16-20.9 years; age class 6: 21-25.9 years; age class 7: 26-30 years. -: not available.
- 771 Abbreviations: F, females; M, males; N, number of individuals; Femoral\_length, maximum length of
- the femur; Femoral\_diameter, transverse diameter at midshaft of the femur; GM\_length, entheseal
- 1773 length of *gluteus maximus*; GM\_width, entheseal width of *gluteus maximus*; Tibial\_length, maximum
- 174 length of the tibia; Tibial\_diameter, transverse diameter at midshaft of the tibia; SOL\_length,

entheseal length of *soleus*; SOL\_width, entheseal width of *soleus*. W: Wilcoxon rank-sum test calculated on the linear measurements of the femur and GM enthesis (left) and tibia and SOL enthesis (right) by sex within age classes; \*: p < 0.05, \*\*: p < 0.01, \*\*\*: p < 0.001 and \*\*\*\*: p < 0.0001; NS: nonsignificant result.

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## 780 Figure S1. FAMDs relating to GM (a) and SOL (b) entheses divided sex.

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- *Note*: The points indicating the centroids of the ellipses are wider and have a colored outline.

Abbreviations: F, females; M, males. GM\_morph (GM1, 2a, 2b, 3 and 1a, 1b, 1c, 2, 3Mariotti), morphological classes of *gluteus maximus*; SOL\_morph (SOL1, 2, 3, 4 and 1a, 1b, 1c, 2, 3Mariotti), morphological classes of *soleus*. Femoral\_closure and Tibial\_closure (0.Fem/0.Tib: unfused epiphyses, 1.Fem/1.Tib: partial state of closure, 2.Fem/2.Tib: total state of closure), degree of epiphyseal closure of the femur and tibia, respectively. Class1, age class 1 (<1 year); Class2, age class 2 (1-5.9 years); Class3, age class 3 (6-10.9 years); Class4, age class 4 (11-15.9 years); Class5, age class 5 (16-20.9 years); Class6, age class 6 (21-25.9 years); Class7, age class 7 (26-30 years).

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- Figure S2. Scree plots and diagrams of the contributions of the variables to Dimensions 1 and 2 of
  the FAMD relating to GM (a, b, c) and SOL (d, e, f) entheses.

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Abbreviations: GM\_morph, morphological classes of *gluteus maximus*; SOL\_morph, morphological classes of *soleus*; Femoral\_length, maximum length of the femur; Femoral\_diameter, transverse diameter at midshaft of the femur; GM\_length, entheseal length of *gluteus maximus*; GM\_width, entheseal width of *gluteus maximus*; Tibial\_length, maximum length of the tibia; Tibial\_diameter, transverse diameter at midshaft of the tibia; SOL\_length, entheseal length of *soleus*; SOL\_width, entheseal width of *soleus*; Femoral\_closure, degree of epiphyseal closure of the femur; Tibial\_closure, degree of epiphyseal closure of the tibia.

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Tables

#### 

Table 1. Distribution by sex and age of the sample.

Age class	Years	F	Μ	Total
1	<1	20	28	48
2	1-5.9	13	9	22
3	6-10.9	2	3	5
4	11-15.9	-	4	4
5	16-20.9	7	11	18
6	21-25.9	6	9	15
7	26-30	5	2	7
То	tal	53	66	119

- Note. -: not available.
- 813 Abbreviations: F, females; M, males.

852 Table 2. Morphological standard for GM (top) and SOL (bottom) entheses.

GM_morph <sup>1</sup>	Description
GM1	The enthesis is defined by a diffuse dense <i>fine porosity</i> (Figure 1).
GM2	The enthesis is defined by a <i>furrowed surface</i> (Figure 2). If the enthesis is mainly characterized (for more than 50% of its surface) by furrows arranged neatly between each other and oriented longitudinally with respect to the length of the diaphysis, assign the morphological subclass <b>GM2a</b> (Figure 2, top); if, on the other hand, the enthesis is characterized mainly (for more than 50% of its surface) by regions in which the furrows have a disordered disposition, then assign the subclass <b>GM2b</b> (Figure 2, bottom). In cases where a mixed morphology occurs, neatly oriented furrows are usually found proximally, while messily oriented furrows are more commonly found distally.
GM3	The enthesis is characterized by a mixed morphology: the same enthesis shows regions with <i>diffuse cortical irregularity</i> and/or a <i>longitudinal protrusion</i> and others with a rather smooth surface defined by a <i>fine porosity</i> or smooth <i>furrows</i> . The <i>mineralized tissue formations</i> can generally be found from the proximal border and along the medial border of the enthesis, while the finely porous smooth surface is generally prevalent at the distal and lateral border, thus tracing a proximal-distal pattern. The <i>mineralized tissue formations</i> can be covered by a layer of <i>woven bone</i> , either continuously or discontinuously (Figure 3).
SOL_morph <sup>2</sup>	Description
SOL1	The enthesis is totally indistinguishable, there is no type of morphological discontinuity between the entheseal area and the surrounding bone (Figure 4).
SOL2	The inferolateral border of the triangular surface covered by the <i>popliteus</i> muscle is sharply defined by the soleal line, which separates an area of porous-looking bone (i.e., the triangular "popliteal" surface – Cunningham et al., 2016, pp: 415–416) from the cortical surface distal to it. The enthesis appears only as a line with no width (Figure 5).
SOL3	The enthesis is defined by <i>furrowed surface</i> with short and shallow furrows. The furrows may enclose oval- shaped pores (Figure 6).
SOL4	The enthesis is characterized by a mixed morphology: the same enthesis shows regions with <i>diffuse cortical irregularity</i> and/or a <i>longitudinal protrusion</i> and others with a <i>finely porous/furrowed surface</i> . The two morphologies coexist on the same enthesis and can appear discontinuously along it, but the <i>mineralized tissue formations</i> occur more frequently in the distal portion of the enthesis, while the porotic/furrowed surface proximally, thus tracing a distal-proximal pattern. The <i>mineralized tissue formations</i> can be covered by a layer of <i>woven bone</i> , either continuously or discontinuously (Figure 7).
Note. If more m <sup>1</sup> : All juvenile C a fossa, the bord GM3 the minera those typical of <sup>2</sup> : The trace of th half. In younger popliteal surface classes SOL2, S same enthesis. Abbreviations: C	ore than 50% of the enthesal surface is damaged the evaluation must be considered nonrecordable (NR). M morphologies can be either on a flat surface or in a fossa; in some cases, especially when the enthesis presents ders of the enthesis can be angled and well evident and should not be confused with a longitudinal protrusion. In <i>ulized tissue formations</i> never affect the entire surface of the enthesis, and this distinguishes this morphology from adults (Mariotti et al., 2007). e SOL enthesis is often discontinuous and there are areas where the trace is totally absent, most often in its proximal individuals, the proximal portion of the enthesis can be indistinguishable among the porosity that characterizes the In these cases, the morphologic assessment must necessarily be performed in its visible portion. In morphological OL3 and SOL4 a fossa can be present, which can be constituted by a single or more different sunken areas on the GM_morph, morphological classes of <i>gluteus maximus</i> ; SOL_morph, morphological classes of <i>soleus</i> .

 $\begin{array}{c} 853\\ 854\\ 855\\ 856\\ 857\\ 858\\ 859\\ 860\\ 861\\ 862\\ 863\\ 864\\ 865\\ 866\\ 866\\ 867\\ 868\\ 869\\ 870\\ 871\\ 872\\ 873\\ 874\\ 875 \end{array}$ 

**Table 3.** Intraobserver and interobserver error results

	Intra	aobserver error	Interobserver error			
Morphological standards	$\kappa^{a}$	Accuracy	κa	Accuracy		
GM_morph	0.93	95%	0.21	65%		
SOL_morph	0.98	95%	0.90	75%		
Linear entheseal measurements	ICC <sup>b</sup>		ICC <sup>b</sup>			
GM_length	0.98		0.93			
GM_width	0.93		0.81			
SOL_length	0.97		0.55			
SOL_width	0.87		0.53			

*Note*. <sup>*a*</sup>: Cohen kappa coefficient. <sup>*b*</sup>: Intraclass correlation coefficient.

Abbreviations: GM\_morph, morphological classes of *gluteus maximus*; SOL\_morph, morphological classes of *soleus*; GM\_length, entheseal length of *gluteus maximus*; GM\_width, entheseal width of *gluteus maximus*; SOL\_length, entheseal length of *soleus*; SOL\_width, entheseal width of *soleus*.

926 Table 4. Subdivision of the individuals by the different morphological classes of GM (top) and SOL (bottom) entheses they have been assigned to, with the distinction en sex and age class.

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	CM momb		class 1	Age	class 2	Age	class 3	Age	class 4	Age	class 5	Age	class 6	Age	class 7			Total
	GM_morpn	F	Μ	F	Μ	F	Μ	F	Μ	F	Μ	F	Μ	F	Μ	F	Μ	F+M
GM1		1	-	5	2	2	3	-	3	4	5	-	3	-	-	12	16	28 (23.5%)
GM2a		12	25	-	-	-	-	-	1	-	-	-	-	-	-	12	26	38 (31.9%)
GM2b		7	3	8	7	-	-	-	-	-	-	-	-	-	-	15	10	25 (21.0%)
GM3		-	-	-	-	-	-	-	-	3	4	2	3	1	-	6	7	13 (10.9%)
1a Mariott	i	-	-	-	-	-	-	-	-	-	1	1	-	-	-	1	1	2 (1.7%)
1b Mariott	i	-	-	-	-	-	-	-	-	-	-	1	-	1	-	2	-	2 (1.7%)
1c Mariott	i	-	-	-	-	-	-	-	-	-	-	2	2	2	-	4	2	6 (5.0%)
2 Mariotti		-	-	-	-	-	-	-	-	-	1	-	-	1	1	1	2	3 (2.5%)
3 Mariotti		-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	2	2 (1.7%)
Total		20	28	13	9	2	3	-	4	7	11	6	9	5	2	53	66	119
E tost	by sex <sup>NS</sup> , by age class ***																	
r test	by sex within age classes		*		NS		NS		-		NS		NS		NS			
	9			-						-				-				
	SOL mornh	Age	class 1	Age	class 2	Age	class 3	Age	class 4	Age	class 5	Age	class 6	Age	class 7			Total
	SOL_morph	Age of F	class 1 M	Age o F	class 2 M	Age F	class 3 M	Age F	class 4 M	Age F	class 5 M	Age F	class 6 M	Age F	class 7 M	F	М	Total F+M
SOL1	SOL_morph	<b>Age</b> <b>F</b>	class 1 M 5	Age o F	class 2 M	Age F	class 3 M	Age F	class 4 M	Age F	class 5 M	Age F	class 6 M	Age F	class 7 M	<b>F</b>	<u>М</u> б	<b>Total</b> <b>F+M</b> 7 (5.9%)
SOL1 SOL2	SOL_morph	<b>Age</b> <b>F</b> 1 4	class 1 <u>M</u> 5 8	Age o F -	class 2 <u>M</u> 1	Age F -	class 3 M -	Age F -	class 4 M -	Age F -	class 5 <u>M</u>	Age F -	class 6 M -	Age F -	class 7 M -	<b>F</b> 1 4	<b>M</b> 6 8	<b>Total</b> <b>F+M</b> 7 (5.9%) 12 (10.1%)
SOL1 SOL2 SOL3	SOL_morph	Age of <b>F</b> 1 4 15	class 1 M 5 8 15	Age o F - 13	class 2 <u>M</u> 1 - 8	Age F - 2	class 3 <u>M</u> - 3	Age F - -	class 4 <u>M</u> - 1	Age F - 4	class 5 <u>M</u> - 2	Age - - -	class 6 M - -	Age F - -	class 7 M - -	<b>F</b> 1 4 34	M 6 8 29	<b>Total</b> <b>F+M</b> 7 (5.9%) 12 (10.1%) 63 (52.9%)
SOL1 SOL2 SOL3 SOL4	SOL_morph	Age 6 F 1 4 15 -	Class 1 M 5 8 15 -	Age o F - 13 -	class 2 <u>M</u> 1 - 8 -	Age F - 2 -	class 3 <u>M</u> - - 3 -	Age F - - -	class 4 <u>M</u> - 1 3	Age F - 4 3	class 5 <u>M</u> - 2 7	Age F - - 3	class 6 <u>M</u> - - 4	Age F - - 1	class 7 <u>M</u> - - -	<b>F</b> 1 4 34 7	<b>M</b> 6 8 29 14	<b>Total</b> <b>F+M</b> 7 (5.9%) 12 (10.1%) 63 (52.9%) 21 (17.6%)
SOL1 SOL2 SOL3 SOL4 1a Mariott	SOL_morph	Age of F 1 4 15 -	class 1 <u>M</u> 5 8 15 -	Age o F - 13 -	class 2 <u>M</u> 1 - 8 -	Age F - 2 -	class 3 <u>M</u> - - 3 -	Age F - - - -	class 4 <u>M</u> - 1 3 -	Age F - 4 3 -	class 5 <u>M</u> - 2 7 -	Age F - - 3 -	class 6 <u>M</u> - - 4 -	Age F - - 1 1	class 7 <u>M</u> - - - -	<b>F</b> 1 4 34 7 1	<b>M</b> 6 8 29 14	<b>Total</b> <b>F+M</b> 7 (5.9%) 12 (10.1%) 63 (52.9%) 21 (17.6%) 1 (0.8%)
SOL1 SOL2 SOL3 SOL4 1a Mariott 1b Mariott	SOL_morph i	Age of F 1 4 15 - -	class 1 <u>M</u> 5 8 15 - - -	Age o F - 13 - -	2 class 2 M 1 - 8 - -	Age F - 2 - -	class 3 <u>M</u> - - 3 - -	Age F - - - -	class 4 <u>M</u> - 1 3 - -	Age F - 4 3 -	class 5 <u>M</u> - 2 7 -	Age F - - 3 - 1	class 6 <u>M</u> - - 4 - 1	Age F - - 1 1 -	class 7 <u>M</u> - - - - - -	<b>F</b> 1 4 34 7 1 1	<b>M</b> 6 8 29 14 - 1	<b>Total</b> <b>F+M</b> 7 (5.9%) 12 (10.1%) 63 (52.9%) 21 (17.6%) 1 (0.8%) 2 (1.7%)
SOL1 SOL2 SOL3 SOL4 1a Mariott 1b Mariott 1c Mariott	SOL_morph i i i	Age ( F 1 4 15 - - - -	class 1 <u>M</u> 5 8 15 - - - -	Age o F - 13 - -	class 2 <u>M</u> 1 - 8 - -	Age F - 2 - - - -	class 3 <u>M</u> - - 3 - - - -	Age F - - - - - -	class 4 <u>M</u> - 1 3 - - -	Age F - 4 3 - -	class 5 <u>M</u> - 2 7 - 1	Age F - - 3 - 1 2	class 6 <u>M</u> - - 4 - 1 -	Age F - - 1 1 - 2	class 7 <u>M</u> - - - - - - - -	<b>F</b> 1 4 34 7 1 1 4	<b>M</b> 6 8 29 14 - 1 1	Total           F+M           7 (5.9%)           12 (10.1%)           63 (52.9%)           21 (17.6%)           1 (0.8%)           2 (1.7%)           5 (4.2%)
SOL1 SOL2 SOL3 SOL4 1a Mariott 1b Mariott 1c Mariott 2 Mariotti	SOL_morph i i i	Age ( F 1 4 15 - - - - - -	class 1 <u>M</u> 5 8 15 - - - - - - -	Age o F - 13 - - - - - -	Class 2 M 1 - 8 - - - - -	Age F - 2 - - - -	class 3 <u>M</u> - - 3 - - - - - -	Age F - - - - - - -	class 4 <u>M</u> - 1 3 - - - -	Age F - 4 3 - -	class 5 <u>M</u> - 2 7 - 1 1	Age F - - 3 - 1 2 -	class 6 <u>M</u> - - 4 - 1 - 4 - 4	Age F - - 1 1 - 2 1	class 7 <u>M</u> - - - - - - 1	<b>F</b> 1 4 34 7 1 1 4 1	<b>M</b> 6 8 29 14 - 1 1 6	Total           F+M           7 (5.9%)           12 (10.1%)           63 (52.9%)           21 (17.6%)           1 (0.8%)           2 (1.7%)           5 (4.2%)           7 (5.9%)
SOL1 SOL2 SOL3 SOL4 1a Mariott 1b Mariott 1c Mariott 2 Mariotti 3 Mariotti	SOL_morph i i i	Age ( F 1 4 15 - - - - - - -	class 1 M 5 8 15 - - - - - - - - - -	Age o F - - - - - - - - - - - - -	Class 2 M 1 - 8 - - - - - - -	Age F - - - - - - -	class 3 M - - 3 - - - - - -	Age F - - - - - - - - -	class 4 <u>M</u> - 1 3 - - - - - - -	Age F - - - - - - - - - -	class 5 <u>M</u> - 2 7 - 1 1 1	Age F - - 3 - 1 2 - -	class 6 <u>M</u> - - 4 - 1 - 4 - 4	Age F - - 1 1 - 2 1 -	class 7 <u>M</u> - - - - 1 1	<b>F</b> 1 4 34 7 1 1 4 1 -	M 6 8 29 14 - 1 1 6 1	Total           F+M           7 (5.9%)           12 (10.1%)           63 (52.9%)           21 (17.6%)           1 (0.8%)           2 (1.7%)           5 (4.2%)           7 (5.9%)           1 (0.8%)
SOL1 SOL2 SOL3 SOL4 1a Mariott 1b Mariott 1c Mariott 2 Mariotti 3 Mariotti <b>Total</b>	SOL_morph i i	Age of F 1 4 15 - - - - 20	class 1 M 5 8 15 - - - - 28	Age o F - - - - - - - - - - - - -	Class 2 M 1 - 8 - - - - - - - - 9	Age F - - 2 - - - - - - 2	class 3 M - - 3 - - - - 3	Age F - - - - - - - - - -	class 4 M - - 1 3 - - - - 4	Age F - - 4 3 - - - - 7	class 5 <u>M</u> - 2 7 - 1 1 1 - 11	Age F - - 3 - 1 2 - - 6	class 6 <u>M</u> - - 4 - 1 - 4 - 2 9	Age F - - 1 1 - 2 1 - 5	class 7 <u>M</u> - - - - 1 1 2	<b>F</b> 1 4 34 7 1 1 4 1 - <b>53</b>	M 6 8 29 14 - 1 1 6 1 66	Total           F+M           7 (5.9%)           12 (10.1%)           63 (52.9%)           21 (17.6%)           1 (0.8%)           2 (1.7%)           5 (4.2%)           7 (5.9%)           1 (0.8%)           1 (0.8%)
SOL1 SOL2 SOL3 SOL4 1a Mariott 1b Mariott 1c Mariott 2 Mariotti 3 Mariotti Total E test	SOL_morph i i by sex <sup>NS</sup> , by age class ***	Age of F 1 4 15 - - - 20	class 1 M 5 8 15 - - - - 28	Age o F - - - - - - - - - - - - -	2 M 1 - 8 - - - - 9	Age F - - 2 - - - - 2	class 3 M - - 3 - - - - 3 3	Age F - - - - - - - - - -	class 4 M - - 1 3 - - - - 4	Age F - - 4 3 - - - 7	class 5 <u>M</u> - 2 7 - 1 1 - 11	Age F - - 3 - 1 2 - 6	class 6 <u>M</u> - - 4 - 1 - 4 - 9	Age F - - 1 1 - 2 1 - 5	class 7 <u>M</u> - - - - 1 1 2	<b>F</b> 1 4 34 7 1 1 4 1 - <b>53</b>	M 6 8 29 14 - 1 1 6 1 <b>66</b>	Total           F+M           7 (5.9%)           12 (10.1%)           63 (52.9%)           21 (17.6%)           1 (0.8%)           2 (1.7%)           5 (4.2%)           7 (5.9%)           1 (0.8%)           1 (0.8%)

Note. Age class 1: <1 year; age class 2: 1-5.9 years; age class 3: 6-10.9 years; age class 4: 11-15.9 years; age class 5: 16-20.9 years; age class 6: 21-25.9 years; age class

7: 26-30 years. -: not available.

Abbreviations: F, females; M, males; GM\_morph, morphological classes of gluteus maximus; SOL\_morph, morphological classes of soleus. F test: Fisher's exact test

928 929 930 931 932 of independence calculated for the morphological classes of GM (top) and SOL (bottom) entheses by sex, age classes and sex within the single age classes; \*: p < 0.05, \*\*: *p* < 0.01, \*\*\*: *p* < 0.001 and \*\*\*\*: *p* < 0.0001; NS: nonsignificant result.

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Table 5. Descriptive statistical analysis (mean and standard deviation) for linear measurements of the femur and GM enthesis (left)

934 and tibia and SOL enthesis (right) with the distinction between sex and age class (Extended version available on Table S1, 935 Supporting Information). All values are expressed in mm.

	F		M			W			F		М			W	
FEMUR	Ν	Mean	SD	Ν	Mean	SD	test	TIBIA	Ν	Mean	SD	Ν	Mean	SD	test
Age class 1								Age class 1							
Femoral_length	6	84.7	31.7	13	67.5	7.9	NS	Tibial_length	9	58.8	26.0	12	61.8	12.9	NS
Femoral_diameter	20	7.4	1.9	27	6.2	1.5	**	Tibial_diameter	20	6.5	1.5	25	5.6	1.1	*
GM_length	8	24.4	5.7	19	20.0	4.5	NS	SOL_length	1	16.0	-	1	14.8	-	-
GM_width	19	3.2	0.8	28	3.0	0.6	NS	SOL_width	8	1.3	0.4	10	1.3	0.5	NS
Age class 2								Age class 2							
Femoral_length	6	165.5	28.9	3	176.0	50.0	NS	Tibial_length	5	121.2	24.7	4	141.6	33.5	NS
Femoral_diameter	12	11.4	1.9	9	11.5	1.9	NS	Tibial_diameter	11	10.1	2.0	9	10.4	1.6	NS
GM_length	9	39.5	6.4	9	34.4	6.3	NS	SOL_length	4	32.1	5.3	4	29.0	11.8	NS
GM_width	13	4.9	1.3	9	5.2	1.3	NS	SOL_width	5	2.6	0.7	4	2.3	0.8	NS
Age class 3								Age class 3							
Femoral_length	2	267.5	2.1	-	-	-	-	Tibial_length	1	225.0	-	1	197.0	-	-
Femoral_diameter	2	16.5	-	2	15.8	1.6	-	Tibial_diameter	1	15.4	-	2	15.4	0.3	-
GM_length	2	57.8	13.9	2	65.1	7.6	-	SOL_length	-	-	-	-	-	-	-
GM_width	2	7.3	1.3	2	7.6	0.5	-	SOL_width	-	-	-	1	2.5	-	-
Age class 4								Age class 4							
Femoral_length	-	-	-	3	312.3	50.9	-	Tibial_length	-	-	-	3	255.0	40.5	-
Femoral_diameter	-	-	-	4	19.7	2.6	-	Tibial_diameter	-	-	-	4	18.8	4.0	-
GM_length	-	-	-	4	78.2	13.4	-	SOL_length	-	-	-	-	-	-	-
GM_width	-	-	-	4	7.8	1.6	-	SOL_width	-	-	-	2	4.8	2.6	-
Age class 5								Age class 5							
Femoral_length	6	404.2	16.8	10	439.6	18.4	**	Tibial_length	6	331.7	8.6	11	364.3	20.1	**
Femoral_diameter	7	22.3	1.5	11	25.9	2.9	**	Tibial_diameter	7	19.1	1.7	10	23.1	2.2	***
GM_length	7	88.2	8.4	7	105.5	17.7	*	SOL_length	5	80.0	17.0	7	86.5	13.0	NS
GM_width	7	8.5	1.3	11	9.4	1.7	NS	SOL_width	6	4.3	0.9	10	5.6	1.0	*
Age class 6								Age class 6							
Femoral_length	6	419.7	9.5	9	459.0	22.5	***	Tibial_length	6	341.5	9.6	9	376.0	24.7	**
Femoral_diameter	6	23.7	2.4	9	27.7	1.8	**	Tibial_diameter	6	21.7	3.4	9	24.8	5.5	NS
GM_length	6	99.3	10.3	7	107.3	8.5	NS	SOL_length	6	78.6	12.7	7	101.5	13.8	*
GM_width	6	7.5	1.01	9	9.9	2.0	*	SOL_width	6	4.3	0.9	9	5.6	1.0	*
Age class 7								Age class 7							
Femoral_length	5	423.8	15.1	2	452.5	51.6	-	Tibial_length	4	339.0	22.1	2	392.0	41.0	-
Femoral_diameter	5	23.9	0.8	2	29.3	2.05	-	Tibial_diameter	5	19.7	1.4	2	23.3	1.1	-
GM_length	3	102.6	4.4	2	107.9	3.0	-	SOL_length	3	100.7	16.7	2	96.6	12.0	-
GM_width	5	8.8	1.0	2	9.1	2.3	-	SOL_width	3	4.63	0.3	2	6.1	2.6	-

Note. Age class 1: <1 year; age class 2: 1-5.9 years; age class 3: 6-10.9 years; age class 4: 11-15.9 years; age class 5: 16-20.9 years; age class 6: 21-25.9 years; age class 7: 26-30 years. -: not available.

936 937 938 939 Abbreviations: F, females; M, males; N, number of individuals; Femoral\_length, maximum length of the femur; Femoral\_diameter, transverse diameter at midshaft of the femur; GM\_length, entheseal length of gluteus maximus; GM\_width, entheseal width of 939 940 941 942 943 gluteus maximus; Tibial\_length, maximum length of the tibia; Tibial\_diameter, transverse diameter at midshaft of the tibia; SOL\_length, entheseal length of soleus; SOL\_width, entheseal width of soleus. W: Wilcoxon rank-sum test calculated on the linear measurements of the femur and GM enthesis (left) and tibia and SOL enthesis (right) by sex within age classes; \*: p < 0.05, \*\*: p< 0.01, \*\*\*: p < 0.001 and \*\*\*\*: p < 0.0001; NS: nonsignificant result.

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Table 6. Spearman correlation and linear regression between the morphological classes of GM (top) and SOL (bottom) and the following variables: age, linear measurements of the bones and entheses and degree of epiphyseal closure, with the distinction between the sexes.

	GM_morph											
FEMUR	]	F	Ν	M	F+M							
	ho <sup>a</sup>	<i>r</i> <sup>2 b</sup>	ho <sup>a</sup>	$r^{2 b}$	ho <sup>a</sup>	<i>r</i> <sup>2 b</sup>						
Age	0.942****	0.795****	0.916****	0.652****	0.933****	0.710****						
Femoral_length	0.868****	0.595****	0.820****	0.526****	0.798****	0.548****						
Femoral_diameter	0.923****	0.714****	0.913****	0.646****	0.906****	0.655****						
GM_length	0.853****	0.593****	0.902****	0.602****	0.888****	0.605****						
GM_width	0.868****	0.581****	0.855****	0.553****	0.848****	0.548****						
Femoral_closure	0.814****	0.689****	0.795****	0.585****	0.799****	0.634****						
			SOL_	_morph								
TIBIA	]	F	Ν	M	F+M							
	$\rho^{a}$	<i>r</i> <sup>2 b</sup>	ho <sup>a</sup>	<i>r</i> <sup>2 b</sup>	$\rho^{a}$	<i>r</i> <sup>2 b</sup>						
Age	0.802****	0.596****	0.852****	0.589****	0.836****	0.579****						
Tibial_length	0.759****	0.415****	0.834****	0.470****	0.819****	0.460****						
Tibial_diameter	0.762****	0.455****	0.852****	0.495****	0.824****	0.485****						
SOL_length	0.695***	0.286*	0.735***	0.439***	0.740****	0.405****						
SOL_width	0.693****	0.287**	0.651****	0.301***	0.674****	0.315****						
Tibial_closure	0.777****	0.622****	0.748****	0.587****	0.753****	0.503****						

*Note*. \*: *p* < 0.05, \*\*: *p* < 0.01, \*\*\*: *p* < 0.001 and \*\*\*\*: *p* < 0.0001.

<sup>a</sup> Spearman's rank correlation (ρ: rho coefficient).

<sup>b</sup> Linear regression model (Adjusted r<sup>2</sup>: coefficient of determination).

969 970 Abbreviations: F, females; M, males; GM\_morph, morphological classes of gluteus maximus; 972 973 974 SOL\_morph, morphological classes of *soleus*; Femoral\_length, maximum length of the femur; Femoral\_diameter, transverse diameter at midshaft of the femur; GM\_length, entheseal length of gluteus maximus; GM\_width, entheseal width of gluteus maximus; Tibial\_length, maximum length of the tibia; Tibial\_diameter, transverse diameter at midshaft of the tibia; SOL\_length, 976 entheseal length of soleus; SOL\_width, entheseal width of soleus; Femoral\_closure, degree of epiphyseal closure of the femur; Tibial\_closure, degree of epiphyseal closure of the tibia.



**Figure 1. Examples of GM1**: fine porosity. **Top left**: BO5 B100 right (F, age class 2), with a fossa. **Top right**: BO6 A337 left (M, age class 3). **Bottom left**: BO50 C4511 right (M, age class 5), with a third trochanter. **Bottom right**: BO98 D6296 right (F, age class 5), with a fossa.



**Figure 2. Examples of GM2**: furrowed surface. **GM2a** on top: neatly oriented furrows; **GM2b** on bottom: randomly oriented furrows. **Top left**: BO29 A298 right (M, age class 1), with a fossa; **Top right**: BO61 B120 left (F, age class 1); **Bottom left**: BO62 B8124 right (F, age class 2), with a fossa; **Bottom right**: BO20 A273 right (M, age class 2), with a fossa.



**Figure 3. Examples of GM3**: mixed morphology formed by mineralized tissue formations and a rather smooth surface defined by a fine porosity or smooth furrows. A proximal-distal pattern of development is recognizable in all four photos, although presenting different degrees of extension of the mineralized tissue. **Top left**: BO25 D5685 right (F, age class 5); **Top right**: BO29 D5921 left (F, age class 6), with a fossa and woven bone covering the mineralized tissue formations; **Bottom left**: BO30 C4564 left (M, age class 6), with a fossa; **Bottom right**: BO51 C4750 left (M, age class 5), with a fossa and woven bone covering the mineralized tissue formations.



**Figure 4. Examples of SOL1**. The enthesis is totally indistinguishable from the surrounding bone. **Top left**: BO4 A213 right (M, age class 2); **Top right**: BO47 A303 left (M, age class 1); **Bottom left**: BO56 A338 right (M, age class 1); **Bottom right**: BO66 A318 left (M, age class 1).



**Figure 5. Examples of SOL2**. The enthesis appears only as a line with no width. **Top left**: BO27 B96 left (F, age class 1); **Top right**: BO33 A296 right (M, age class 1); **Bottom left**: BO40 A324 left (M, age class 1), with a fossa; **Bottom right**: BO45 B101 left (F, age class 1).



**Figure 6. Examples of SOL3**. The enthesis shows a discontinuous surface characterized by\_furrowed surface with furrows that may or may not frame oval-shaped pores. **Top left**: BO3 B8111 left (F, age class 3), with a fossa; **Top right**: BO9 A289 left (M, age class 3), with a fossa, the furrows enclose oval-shaped pores; **Bottom left**: BO55 B8059 left (F, age class 1), with a fossa; **Bottom right**: BO67 A310 right (M, age class 1), the furrows enclose oval-shaped pores.



**Figure 7. Examples of SOL4**. Mixed morphology between a longitudinal protrusion and finely porous/furrowed surface. **Top left**: BO70 D4785 left (F, age class 6), with a fossa and a distal-proximal pattern of development (a layer of woven bone partly covers the longitudinal protrusion); **Top right**: BO99 D5809 left (F, age class 5), with a fossa and woven bone partly covering the longitudinal protrusion; **Bottom left**: BO75 C4848 left (M, age class 6), with a fossa; **Bottom right**: BO96 C1514 right (M, age class 6), with a fossa and a distal-proximal pattern of development (a layer of woven bone fully covers the longitudinal protrusion).



**Figure 8.** Boxplots representing the distribution of the individuals by age within each morphological class assigned to the GM (**top**) and SOL (**bottom**) entheses and divided by sex.

Abbreviations: F, females; M, males.

**Figure 9.** FAMD relating to GM enthesis (**top row**) divided by morphological classes (**a**), sex (**b**), degree of epiphyseal closure of the femur (**c**) and FAMD relating to SOL enthesis (**bottom row**) divided by morphological classes (**d**), sex (**e**), degree of epiphyseal closure of the tibia (**f**). Please refer to the online version of this article for color interpretation.



Abbreviations: F, females; M, males. GM\_morph, morphological classes of *gluteus maximus*; SOL\_morph, morphological classes of *soleus*. Femoral\_closure, degree of epiphyseal closure of the femur; Tibial\_closure, degree of epiphyseal closure of the tibia (0.Fem/0.Tib: unfused epiphyses, 1.Fem/1.Tib: partial state of closure, 2.Fem/2.Tib: total state of closure). Class1, age class 1 (<1 year); Class2, age class 2 (1-5.9 years); Class3, age class 3 (6-10.9 years); Class4, age class 4 (11-15.9 years); Class5, age class 5 (16-20.9 years); Class6, age class 6 (21-25.9 years); Class7, age class 7 (26-30 years).