Elsevier Editorial System(tm) for
International Journal of Paleopathology
Manuscript Draft

Manuscript Number: IJPP-D-15-00108R2

Title: Insights on the paleoepidemiology of ancient tuberculosis from the structural analysis of postcranial remains from the Ligurian Neolithic (northwestern Italy).

Article Type: Full Length Article

Keywords: cross-sectional geometry; ontogeny; robusticity

Corresponding Author: Dr. Vitale Stefano Sparacello, Doctor of Philosophy

Corresponding Author's Institution: Durham University

First Author: Vitale Stefano Sparacello, Doctor of Philosophy

Order of Authors: Vitale Stefano Sparacello, Doctor of Philosophy; Charlotte A Roberts, Doctor of Philosophy; Alessandro Canci, Doctor of Philosophy; Jacopo Moggi-Cecchi, Doctor of Philosophy; Damiano Marchi, Doctor of Philosophy

Abstract: The aim of this research was to gain insights on the timeline of progression of osteoarticular tuberculosis (TB) in people from the Neolithic period by using skeletal traits that are independent of the bony lesions. The body proportions and postcranial mechanical strength of bones from two individuals from Liguria in northwestern Italy (Arene Candide 5, adolescent, and Arma dell'Aquila 1, adult), were compared with the rest of the Ligurian Neolithic skeletal series (45 individuals). If TB led to wasting of the skeleton and lack of normal function which endured for years, as often happens today, a clear signature of postcranial gracility and disruption of development should be apparent. Conversely, rapid progress of the disease would leave little systemic macroscopic change in the skeleton, except for the bony lesions directly caused by the TB pathogen, suggesting a different level of bacterial virulence in the past. The extreme biomechanical gracility observed in the lower limb of Arene Candide 5 suggests a period of compromised diaphyseal periosteal apposition during ontogeny due to metabolic disturbances likely linked to TB. Results suggest that, in Neolithic Liguria, TB in humans saw a slow, chronic progression, which is characteristic of diseases with long histories of host-pathogen co-evolution.

Title: Insights on the paleoepidemiology of ancient tuberculosis from the structural analysis of postcranial remains from the Ligurian Neolithic (northwestern Italy).

Authors: Vitale Stefano Sparacello[1,2], Charlotte A. Roberts[1], Alessandro Canci[3], Jacopo Moggi-Cecchi[4], Damiano Marchi[5]

[1] Department of Archaeology, Durham University, Durham DH1 3LE, United Kingdom.

[2] UMR5199 PACEA, Université de Bordeaux – CNRS, Batiment B8, Allée Geoffroy Saint Hilaire, CS 50023, 33615 Pessac cedex, France.

[3]Department of Archaeology, University of Padua, piazza Capitaniato 7
35139 Padua, Italy

[4] Laboratory of Anthropology, Department of Biology, University of Florence, Florence, Italy

[5] Department of Biology, University of Pisa, Via Derna, 1 56126 Pisa, Italy

Number of figures: 8

Number of tables: 2

Abbreviated title (running headline): Course of Neolithic TB from postcranial analysis

Corresponding author: Vitale Stefano Sparacello, Department of Archaeology, Durham University, Durham DH1 3LE, UK +447478342950

Email: vitale.sparacello@durham.ac.uk; vitosparacello@gmail.com

Abstract

The aim of this research is to gain insights on the progression timeline of osteoarticular tuberculosis (TB) in people from the Neolithic period by using skeletal traits that are independent of the bony lesions. The body proportions and postcranial mechanical strength of bones from two individuals from Liguria in northwestern Italy (Arene Candide 5, adolescent, and Arma dell'Aquila 1, adult), were compared with the rest of the Ligurian Neolithic skeletal series (45 individuals). If TB led to wasting of the skeleton and lack of normal function that endured for years, as often happens today, a clear signature of postcranial gracility and disruption of development should be apparent. Conversely, rapid progress of the disease would leave little systemic macroscopic change in the skeleton, except for the bony lesions directly caused by the TB pathogen, suggesting a different level of bacterial virulence in the past. The extreme biomechanical gracility observed in the lower limb of Arene Candide 5 suggests a period of compromised diaphyseal periosteal apposition during ontogeny due to metabolic disturbances likely linked to TB. Results suggest that, in Neolithic Liguria, TB in humans saw a slow, chronic progression, which is characteristic of diseases with long histories of host-pathogen co-evolution.

Key words: cross-sectional geometry, ontogeny, robusticity.

1. Introduction

In skeletal analyses of ancient disease, in particular tuberculosis (TB), it often has to be assumed that the pathogen acted with a virulence comparable to modern times (i.e. the ability of the organism to invade the tissues of the host and cause disease). The purpose of this paper is to test this assumption by evaluating two Neolithic skeletons (6^{th}-4^{th} millennium BCE) with tuberculous spondylitis from Liguria (Italy). This new approach takes into account changes in skeletal properties that are independent of the osteolytic lesions observed in the skeletons. Through comparisons with the rest of the Neolithic skeletal series from Liguria (Parenti and Messeri, 1962), possible systemic reactions due to chronic TB leading to alterations in normal expected growth are assessed (stature, body mass, and body mass index - BMI), along with the presence or absence of a decrease in the mechanical competence (or 'gracilization') of the postcranial skeleton (evaluated via cross-sectional geometric analysis of the diaphysis, CSG – Ruff et al., 2006). By analyzing systemic changes in bone structure, insights on the timing of progression and virulence of TB in the past are explored to gain new information on the paleoepidemiology of this reemerging disease (Roberts and Buikstra, 2003; http://www.who.int/topics/tuberculosis/en).

Tuberculosis is a mammalian infectious disease caused by bacteria of the *Mycobacterium tuberculosis* complex (MTBC). It is mainly the human and bovine forms that affect humans, which are transmitted via the respiratory and gastrointestinal routes, respectively. The disease has had a long history, as seen in bioarchaeological studies. Skeletal evidence, usually consisting of destructive lesions in the spine (Pott's disease), has been documented in both the Old and New Worlds. Tuberculosis, as seen in human skeletal remains, became more common in Europe as population density increased and people started to live in permanent urban settlements supported by a farming economy. The earliest evidence derives from archaeological sites in Germany, Hungary, Israel, Italy and Poland, with the Israeli evidence dating back to the 8^{th} millennium BCE (see summary in Roberts 2015).

The first Italian skeleton with a diagnosis of TB was reported by Formicola et al. (1987) from Arene Candide, a cave on the Ligurian coast (Fig. 1). Several inhumations from Neolithic

levels have been excavated from caves in the area since the mid-19th century (Del Lucchese, 1997; Maggi 1997a, 1997b, De Pascale, 2008). The skeleton Arene Candide 5 (abbreviated from now on as AC5) was a crouched inhumation, lying on their left side in a stone cist, and directly dated to 7571-7420 cal BP (99.7%; KIA-28340, Le Bras-Goude et al., 2006). Another skeleton with TB in the spine (Arma dell'Aquila 1, abbreviated from now on as AQ1; Canci et al. 1996) was found among the skeletal remains excavated by Zambelli in 1936 (Parenti and Messeri, 1956) at Arma dell'Aquila, a cave in the same area (Arobba et al. 1987; Fig. 1), and was directly dated to 6929-6310 cal BP (99.7%; GrN-17730, Paolo Biagi, personal communication). Individuals AC5 and AQ1 have different ages, the former being an adolescent (about 15 years old; Formicola et al., 1987), and the latter an adult (around 30 years old; Canci et al., 1996). Both individuals display a collapse of the spine due to lytic lesions affecting the lower thoracic and lumbar vertebral bodies. The lesions are typical of tuberculous spondylitis, a form of osteoarticular TB affecting the spine (Spiegel et al., 2005). Individuals who survive spinal TB enter a stage of repair and eventual ankylosis, with re-mineralization of the destroyed vertebrae that often results in a spinal deformity (i.e. kyphosis, Tuli, 2013). Minimal osteoblastic reaction (new bone formation) was noted, suggesting that death occurred while TB was still destroying the vertebral bodies, and therefore that it was still in the active phase.

2. Rationale of the study

Today, the progression of TB is slow and chronic. A recent meta-analysis suggests that the active phase of untreated pulmonary TB from the onset of symptoms to cure or death lasts for, on average, three years, but with considerable variation (Tiemersma et al., 2011). About 90% of modern human TB infections are clinically latent, with the pathogen being contained within granulomatous lesions at the site of the primary infection (Ulrichs et al., 2005). Spinal involvement occurs in only a small fraction of patients with TB (e.g. 1%, Turgut: 2001; 3-5%: Vigorita, 1999; 10% of patients with extra-pulmonary TB: Garg and Somvanshi, 2011), and has been reported to develop within two to three years after primary infection (Girling et al., 1988). Bone changes become apparent after three to five months from initial spinal involvement (Spiegel et al., 2005), with average disease duration ranging from four -11 months to a few years (Garg and Somvanshi, 2011). Compared to pyogenic hematogenous bone diseases, lesions in

skeletal TB develop slowly, with gradual decalcification of the bone and slow 'poisoning' of the cells (El-Najjar, 1981; Meghji et al, 1997)).

The presence of osteoarticular lesions in Neolithic skeletons, if evaluated following modern standards, would therefore suggest a slow and chronic course for the disease. However, it is not safe to model past disease progression based on clinical evidence from patients treated with antibiotics. Changes in the pathogen as well as host immunity may significantly alter TB morbidity and virulence over a relatively short period of time (Palkovich, 1981). In particular, murine models suggest that the chronic slow progression of TB is not due to slow replication of the bacterium itself, but to the ability of the localized immune response to maintain equilibrium and latency (Munoz-Elias et al., 2005; Ulrichs et al., 2005; Gill et al., 2009). There is also clinical evidence suggesting that the host response plays a major role in restricting intracellular mycobacterial growth and therefore in determining the clinical manifestations of the disease (Schluger and Rom, 1998). It is therefore possible that, in Neolithic times, populations with no previous exposure to TB might have had not only a greater susceptibility but also a more rapid negative outcome. It is generally expected that this would result in no traces in the skeleton. However, lack of host resistance could imply a lesser ability to contain the spread of the disease from the lungs to the rest of the body. In the skeleton, this inability to restrict mycobacterial growth would have led to a rapid formation of granulomatous lesions and bone destruction. A similar situation is observable in non-human primates, for whom susceptibility to TB is generally higher than in humans, the acute form is prevalent (Peña and Ho, 2015), and survival time is shorter (Sapolsky and Else, 1987; Isaza, 2003). However, extra-pulmonary lesions, including spinal TB, have been observed in non-human primates (Martin et al., 1968; Fox et al., 1974). The scenario of a rapid-acting, fulminating disease cannot therefore be completely excluded based on the current skeletal evidence.

Since the identification of any disease process affecting skeletal remains is not unequivocal (Palkovich, 1981), paleopathological studies on the epidemiology of TB and its impact on past populations would benefit from independent assessments of disease progression. The current complex pattern of disease containment and reactivation suggests that TB and humans have had a history of host-pathogen coevolution, although consensus has not been yet

reached on when this process began. Depending on the model used to estimate substitution rates, estimates for the coalescence for the MTBC lineages vary of an order of magnitude from thousands to tens of thousands of years (Brosch et al., 2002; Hirsh et al., 2004; Hershberg et al., 2008; Wirth et al., 2008; Comas et al., 2013; Bos et al., 2014). The possibility that TB was a relatively novel disease in Neolithic times would be supported by evidence of a lack of resistance and adaptation of the host to the pathogen. Conversely, if humans had been co-evolving with MTBC for tens of thousands of years, by the Neolithic period TB should be displaying the pattern of chronic progression, latency and reactivation that is characteristic of diseases with long histories of host-pathogen coevolution. It is possible that Neolithic skeletons will therefore show signs of systemic 'wasting' and metabolic disturbances while the disease slowly progressed for years.

In this study, we consider the effects on the skeleton of possible systemic reactions, alterations in normal growth trajectory (stature, body mass, and BMI), and gracilization of the postcranial skeleton (evaluated via CSG), due to the long-term debilitating effect of TB. Tuberculosis causes severe metabolic disturbances (cytokine activation, abnormal protein metabolism, and hormonal unbalance) causing 'consumption' (Macallan, 1999; Schwenk and Macallan, 2000; del Rey et al., 2007). In addition, spinal TB is expected to reduce normal activity (and in some cases leads to immobilization) because of symptoms such as weakness or numbness of the lower limbs (due to spinal nerve damage), severe pain, and signs such as an unsteady or abnormal gait (Luk, 1999), and a psoas abscess (Chandler and Page, 1940; Resnick and Niwayama, 1995), respiratory dysfunction (Aufderheide and Rodríguez Martin 1998), and paraplegia with flaccid paralysis (Turgut, 2001; Spiegel et al., 2005).

While the vertebral lesions directly caused by TB could theoretically have had a more rapid development than in people today, systemic reactions to metabolic disturbances, malnutrition, and immobilization require more time to become apparent in the skeleton. If our results suggest that the skeletons with TB lesions also show signs of stunting or wasting, we could infer that they are due to a long-lasting active TB phase, and an estimate of its duration will be attempted. In fact, finding skeletal properties that are more compatible with a younger age class would provide a rough estimate of when the disease began compromising the

individual's growth. The analysis of two individuals in different ontogenetic phases (AC5, adolescent, and AQ1, around 30 years old), with skeletons that would have had different sensitivities and adaptive responses to metabolic insults, would enable a rough assessment of whether the course of the disease lasted months, years, or several years. The possible systemic changes, as well as a rationale for their interpretation based on the available clinical evidence, are listed below:

2.1. Body size and proportions.

Several studies demonstrate a relationship between early disturbances in growth due to infectious disease and malnutrition, and attained stature (Vercellotti et al., 2014, and references therein) and body proportions (Robbins Schug, 2011). Numerous factors including catch-up growth and selective mortality warn against making simplistic inferences about the environmental conditions of a population from average stature (Wood et al., 1992; Vercellotti et al., 2014). However, contrasting the stature of AC5 with his peers can provide insights on the presence of stunting due to metabolic disturbances. As AQ1 is an adult, it is probable that the infection affected her well after the completion of skeletal development. Nevertheless, short stature has already been noted for this individual (Canci et al., 1996), and therefore a more accurate assessment of possible severe stunting for this individual is necessary.

2.2. Postcranial mechanical rigidity.

In the absence of environmental disturbances, the total and cortical area of long bone cross sections at the age of peak bone mass are the result of the 'mechanical environment' affecting body dimensions and activity levels during the pre- and peri-pubertal periods (Lazenby, 1990; Pearson and Lieberman, 2004; Sparacello and Pearson, 2010). Metabolic insults such as malnutrition significantly alter bone development by slowing down subperiosteal apposition and accelerating medullary expansion (Garn et al., 1964, 1969; Himes et al., 1975). The concomitant effect of altered metabolism and activity pattern in AC5 during a crucial time for bone development is expected to result in extremely low 'robusticity' (bone mechanical rigidity scaled by body size: Ruff et al., 2006) of long bones (humerus, femur, and tibia) when compared to the rest of the Neolithic sample. A decrease in mechanical competence will be particularly influenced by a deficit in subperiosteal apposition, since the total area is the main determinant of

bone rigidity (Stock and Shaw, 2007). As AQ1 is fully adult, long-term metabolic disturbances and inactivity are expected to result in an enlargement of the medullary cavity and decreased percent cortical area (Eser et al., 2004).

2.3. Cross-sectional indices and shape.

Patterns in cross-sectional indices can be informative about subsistence practices (Trinkaus and Ruff, 1999). Ligurian Neolithic people show highly characteristic and sex-specific CSG indices: males have shown asymmetry in mechanically induced robusticity of the humeri, which suggests the frequent use of tools using one hand (maybe axes for woodworking), while females show symmetry, suggesting the use of both arms (possibly querns for cereal processing; Marchi et al., 2006; Sparacello and Marchi, 2008; Sparacello et al., 2011). Furthermore, lower limb CSG shape indices have also been associated with mobility levels (Carlson and Marchi, 2014); Ligurian Neolithic males show high 'mobility indices' in the lower limbs, probably due to the importance of pastoralism among these people (Rowley-Conwy, 1997; Marchi et al., 2006, 2011). Given that participation in subsistence related activities in the Neolithic probably began as early as late childhood (especially herding, as happens cross-culturally; Dyson-Hudson and Dyson-Hudson, 1980; Hobbs et al., 1999), it is expected that some of those postcranial adaptations may be present in adolescents, including AC5. Given the adult status of AQ1, those postcranial adaptations might be expected to be well established, especially the remarkably low humeral bilateral asymmetry, which is a common trait among females. Not finding those postcranial adaptations in both individuals would suggest that TB had affected their lifestyle since late childhood.

It should be noted that, in the case of humeral asymmetry, we are not measuring fluctuating asymmetry due to the direct effect of metabolic disturbances (e.g. Palmer and Strobeck, 1992). Rather, we are assessing whether directional asymmetry was different in the pathological individuals due to the possibility of a lack of performing specific activities typical of the rest of the population.

2.4. Limitations of the model.

The expected outcomes outlined above are based on clinical evidence, but there is currently no comprehensive model predicting in detail how metabolic insults such as

malnutrition, parasitic infection, or disease will affect development of bone length, articular size, cross-sectional periosteal and endosteal dimensions, and other traits not considered here such as bone mineral and trabecular density. In particular, how the various traits interact is unclear (Robbins Schug and Goldman, 2014; Eleazer and Jankauskas, 2016). Some traits may be affected directly by metabolic disturbances, while others may be underdeveloped due to the indirect effect of another change (e.g. arrested growth in length due to a metabolic insult might secondarily result in small cross-sectional areas). However, these forms of positive feedback would only reinforce the outcome of gracility that, based on skeletal lesions, we would attribute to systemic metabolic disturbance due to TB. On the other hand, the interplay between different traits may result in compensatory mechanisms; for example, increased medullary area and cortical porosity due to metabolic insult might result in subperiosteal expansion. However, while this mechanism has been observed in ribs, it does not appear significant for long bones (Eleazer and Jankauskas, 2016), which are the skeletal elements studied here. In the absence of a complete model, we will consider severe deviations from the normal variation of the Neolithic sample in one or more of the traits above as evidence of disease-induced metabolic insult, without attempting to predict in detail which trait will be affected.

Another limitation of the study, which is inherent in most bioarchaeological research, is the impossibility of comparing the individuals with osteoarticular TB with a skeletal sample that is representative of the 'healthy' living population. In addition to the fact that 'health' is not only a matter of absence of lesions (Temple and Goodman, 2014), the well-known problem of the 'Osteological Paradox' is relevant here: we are comparing individuals who died in a certain class of age, and they therefore are probably not representative of the surviving population (Wood et al., 1992). In the case of TB, this problem is even more apparent: it is unlikely that we are comparing infected and non-infected individuals, given that the prevalence of the skeletal form of this infection is very low (1-5% in modern untreated cases, see above). In fact, a significant portion of the people from the comparative sample may have also died from TB, and could have suffered from long-term chronic metabolic alterations due to the disease without showing osteoarticular lesions. Therefore, some individuals in the comparative sample might show systemic gracilization or growth stunting due to TB (or other diseases), although the skeletal

series is on average as robust or more so than modern comparisons and other samples from various periods (Sparacello and Marchi, 2008; Sparacello et al., 2011). However, this issue can be incorporated into the model. Finding that the individuals with osteoarticular TB are more gracile when compared to their peers would suggest that they are the most impaired individuals along a continuum that also affected others, and would still demonstrate that TB had the potential for manifesting as a slow-developing chronic disease. The possibility of having sampled other individuals with systemic gracilization will be discussed if individuals with skeletal TB will be in the lower portion of the range of distribution of the comparative sample. Although the above caveats suggest extreme caution when interpreting the results of this study, we believe that this research framework shows potential for complementing our knowledge about past TB virulence.

3. Materials and Methods

The comparative sample for AC 5 and AQ1 includes all the Ligurian Neolithic skeletons that are complete enough for CSG analysis and do not show clear signs of skeletal pathology (Parenti and Messeri, 1962; Formicola et al., 1987; Benvenuti, 2012; Pagani 2012). The total sample size, including the two individuals with TB, is 45 individuals (14 sub-adults, 20 adult males, and 11 adult females; Table 1). They were excavated from various caves and rock shelters located within a radius of about five kilometers around the Finale Ligure (Liguria, Italy) area (Fig. 1): Arene Candide, 17 individuals; Arma dell'Aquila, four individuals; Bergeggi, four individuals; Boragni, two individuals; Pian del Ciliegio, one individual, and Pollera, 17 individuals. During the Middle Neolithic, all these sites were likely part of the same pastoralism system described for the Arene Candide cave (Maggi and Nisbet, 1990; Rowley-Conwy, 1997). The skeletal remains are curated in four museums: the Civico Museo del Finale (Finale Ligure, Savona), the Museo di Archeologia Ligure (Pegli, Genoa), the Museo di Storia Naturale dell'Università di Firenze (Florence), and the Museo Nazionale Preistorico Etnografico Luigi Pigorini (Rome). Osteometric data from the literature were used for the skeletons curated in Rome (Parenti and Messeri, 1962).

3.1. Age and Sex

Age at death for the juvenile skeletons was estimated using dental development and eruption (Haavikko, 1970; Ubelaker, 1979); for adolescents, the dental data were considered

alongside information about epiphyseal fusion stages of the long bones and pelvis (morphological summaries in Schaefer et al., 2009). The results substantially agree with previous age estimations (Parenti and Messeri, 1962; Formicola et al., 1987; Benvenuti, 2012; Pagani 2012). For two individuals, Arene Candide 6731 and Pollera 5, only the postcranium was available for study. Their age estimation (12-16 years old) is based on epiphyseal fusion. In no case was age calculated only using bone lengths, given the differences in body proportions between Neolithic individuals and modern skeletal samples (Formicola and Franceschi, 1996; Marchi et al., 2011). Among adults, only individuals showing signs of advanced senescence are usually excluded from CSG studies (Ruff et al., 1994; Macintosh et al., 2014). As in previous research (Marchi et al., 2006), adults were included in a single age category, and adult status was determined based on the complete fusion of the long bone epiphyses and eruption of the permanent dentition.

For adult individuals, sex was estimated from pelvic and cranial morphology following the standards listed in Buikstra and Ubelaker (1994, p 15-20), and Bruzek (2002). Recommendations from Schutkowski (1993) were taken into account for adolescents, some of which appear to show traits more compatible with males, particularly a narrow sciatic notch. However, it has been demonstrated that the methods used to assess sex in adolescents have unsatisfactory success rates (Scheuer and Black, 2004:20), and therefore sex attributions in this study should be considered tentative.

3.2. CSG methodology

The protocol used to position, measure, and determine the level of the diaphyseal section of long bones to be measured for CSG analysis of juveniles is detailed in Cowgill (2008, 2010). Periosteal contours were reconstructed via polysiloxane molds or 3D structured-light scanning (DAVID® SLS-2). For periosteal molds, CSG properties were calculated using a version of the program SLICE (Nagurka and Hayes, 1980) adapted as a macro routine inserted in Scion Image release Beta 4.03. The custom-built AsciiSection software (Davies et al., 2012) was used for 3D scans. 'Hollow bone' CSG properties (i.e. taking into account the area of the medullary cavity) were estimated from 'solid' CSG properties using regression equations (Sparacello and Pearson, 2010; Marchi et al., 2011; Davies at al., 2012; Macintosh et al., 2013).

Cross-sectional properties of most of the adult individuals in this study have been analyzed in previous studies (Marchi et al., 2006, 2011; Marchi, 2008; Sparacello and Marchi, 2008; Sparacello et al., 2011, 2014; Table 1). Cross-sectional geometric properties for the recently excavated individual from Pian del Ciliegio (Pian del Ciliegio 1, Del Lucchese, 2014) were estimated using the SolidCSG method. Mid-shaft external diameters from the literature (Parenti and Messeri, 1962) were used to estimate CSG in five individuals using regression equations (Table 1) (Pearson et al., 2006; Pearson and Sparacello, In Press) (Table 1).Overall diaphyseal strength is evaluated in this study using the section modulus (Z_p) calculated as $J^{0.73}$, where J is the polar second moment of area of a cross section (Ruff, 1995, 2000). Z_p was scaled by dividing it by bone mechanical length × body mass (Ruff, 2000).

The articular dimension that best predicts body mass is femoral metaphyseal breadth for non-adult skeletons with estimated ages of between eight and 10 years old. We therefore averaged the formulae from Robbins Schug et al. (2013) and Ruff (2007). Femoral metaphyseal breadth is a poor estimator of body mass, but the diameter of the femoral head is a significant predictor for adolescents and adults, (Ruff, 2007; Robbins Schug et al., 2013). Given that we have an independent predictor of age from dental maturation, Ruff's (2007) age-specific formulae for the estimated upper and lower age boundaries were employed, and the results were averaged. Adult body mass was estimated based on the superoinferior diameter of the femoral head following the guidelines in Trinkaus and Ruff (2012).

Non-adult stature was estimated from femoral diaphyseal length following Ruff (2007), except for the Bergeggi 1 individual where the tibia was used due to the postmortem absence of the distal femoral epiphysis. Results from the estimated upper and lower age boundaries were averaged. Formicola and Franceschi's equations (1996) were used for adults, which are the most appropriate for Early Holocene samples. The body mass index (BMI) was calculated as [body mass (kg) / stature^2 (m^2)] following Robbins Schug (2011).

Non-standardized J was used to explore humeral bilateral asymmetry using the formula $[(J_{max}-J_{min})/J_{min}] \times 100$, following previous research (Rhodes and Knüsel, 2005). Diaphyseal shape in CSG refers to the ratios of (non-standardized) second moment of areas (SMAs), which are proportional to bending rigidity. Following previous studies, I_{max}/I_{min} (the ratio of the

maximum and minimum SMA) was used for the tibia, while I_x/I_y (the ratio of SMAs calculated about ML and AP planes) was used for the femur (Sparacello et al., 2014).

It should be noted that calculating CSG properties from periosteal contours might slightly underestimate J in adolescents. Due to greater periosteal than endosteal development during growth, relative cortical area normally increases during adolescence and early adulthood (Garn, 1970; Ruff et al., 1994; Ruff et al., 2013). In young adults, the combined effect of growth and mechanical loading can lead to medullary stenosis (Ruff et al., 1994). However, given that an attempt to detect major deviations in bone rigidity in specific individuals due to disease is being made, a slight underestimation of the effect of possible medullary stenosis should not significantly affect the results.

Evaluations of the mechanical competence of individuals with signs of disease, especially when the pathology involves bone resorption, as in the case of TB, cannot be exempt from an assessment of the extension of the medullary cavity (Ruff et al., 1994; Ruff, 2010). Cortical area was plotted against the total area of the section for the adult individual AQ1 and compared with the individuals for whom these data were available. Only antero-posterior radiographs of both tibiae were available for the adolescent AC5. Therefore, the sum of the medial and lateral thickness of the cortical bone at the midshaft of the tibia was plotted against the diameter at the same level, and compared with the data from the adult sample. A similar method, but based on femoral medio-lateral radiographs, was adopted from previous research (Mays et al., 2009).

3.3. Statistical analysis.

Differences in body proportions and CSG properties between AC5, AQ1, and the comparative samples of juvenile and adult skeletons were visualized via individual value plots. The differences between individual values for the two individuals with TB and sample statistics were quantified by considering as 'significantly low' a value below two standard deviations from the mean of their reference sample (adults for AQ1, and adolescents 12-16 years old for AC5), or below the 10^{th} percentile (for AQ1 only, given the small sample size of adolescents). Percentiles are commonly used as a descriptive tool to detect deviations in BMI from the norm (Whitaker et al., 1997). Statistical analyses were performed using Statistica 10 (Statsoft, 2011) and Minitab 16 (Minitab, 2010).

4. Results

4.1. Body size and proportions

Table 2 shows individual values for the variables included in this study for AC5, AQ1 and the comparative adolescent sample, along with sample statistics of the adult comparative sample composed of individuals not showing skeletal signs of TB. As already noted in previous studies when compared to females, adult males show a significantly greater stature and body mass, also displaying significantly more robust humeri and tibiae (e.g. Marchi et al., 2006, 2011; Sparacello and Marchi, 2008). Moreover, males have significantly higher femoral and tibial shape indices, as well as a higher degree of humeral bilateral asymmetry.

When comparing the individuals with TB with the non-adult comparative sample, AC5 displays the lowest values for stature and body mass in his age-class (12-16 years old), although they do not appear to greatly deviate from the growth curve constructed using the available non-adult skeletal remains (Fig. 2). AQ1 falls among the shortest and lightest of the Neolithic females, but is what appears to be within the normal variation for the adult sample.

The BMI in the sample increases with age and plateaus towards adulthood (Fig. 3), a trend that is similar to that observed in modern samples (Kuczmarski et al., 2002). AC5 and AQ1 are within normal variation. It can be noted that BMI for some adults is quite high, falling above the 90^{th} percentile for contemporary Americans (Kuczmarski et al., 2002), in agreement with the observation that Ligurian Neolithic people, especially males, have stockier body proportions than contemporary people (Marchi et al., 2011).

4.2. Postcranial mechanical rigidity and cross-sectional indices

Figure 4 shows the growth curve of non-standardized section moduli (Z_p), for the humeri, femora, and tibiae. The growth curve obtained here can be compared with the longitudinal study of Ruff (2003a, Fig. 1). In both studies, a three- to four-fold increase in femoral rigidity from the age of 8-10 years to adulthood (or average adult Z_p) can be observed. The non-adults included in the sample provide, therefore, a reasonably realistic estimate of the ontogenetic trajectory of long bone mechanical competence for Ligurian Neolithic people.

In Figure 5, the growth curves for Z_p scaled by body size of the humeri, femur, and tibia of the sample show a decline from childhood to adolescence and adulthood. This appears a

realistic trend, because it parallels that observed in Ruff's longitudinal study of the humerus and femur, including the steeper decrease with age for humeral Z_p when compared to femoral Z_p (Ruff, 2003a, Fig. 3; Ruff, 2003b, Fig. 9).

When bone rigidity is standardized, AC5 displays the lowest value for his age-class only for the lower limb (Figure 5). However, only the mechanical strength of the femur is below two standard deviations the mean of the adolescent sample (Table 2). The results for standardized Z_p of AQ1 fall within the normal variation for Neolithic females (Fig. 5), and although they are in the lower part of the distribution, they are neither below two standard deviations of the mean, nor below the 10^{th} percentile of the female adult sample (Table 2).

The plot of midshaft tibial cortical thickness (medial + lateral thickness measured from antero-posterior (AP) radiographs: Fig. 6) against its diameter shows a significant positive correlation ($r=0.76$, $P<0.001$). Although the predictive power is not high ($r^2=0.58$), it is apparent that AC5 does not deviate greatly from the regression line. Therefore, no cortical thinning is obvious in AC5. The correlation between humeral and femoral cortical and total area is stronger, and holds higher predictive power (right humerus: $r=0.94$, $P<0.001$, $r^2=0.88$; left humerus: $r=0.9$, $P<0.001$, $r^2=0.80$; femur: $r=0.94$, $P<0.001$, $r^2=0.88$). AQ1 falls above the regression line, and therefore does not show cortical thinning for both humeri and the femur.

4.3. Cross-sectional indices and shape.

Humeral bilateral asymmetry for AC5 (9.72%) falls within normal variation, and AQ1 shows a value (4%) that is close to the mean of the Neolithic female sample (5.33% Table 2). The femoral shape index (I_x/I_y) for AC5 is 1.48, falling in the higher part of the adult male variance, which appears to be common in the adolescents age-class (Fig.7, and Table 2). Although adult values show considerable variation, female femoral shape indices tend to be lower than those of males (Table 2). AQ1 (together with another female individual, Pollera 14) shows the lowest value in the female sample, at 0.96. In contrast with femoral shape, adult values of tibial shape (I_{max}/I_{min}) are reached only in late adolescence/early adulthood. AC5 does not seem to deviate from the growth curve, while AQ1 is within adult female range of variation (Fig. 7, and Table 2).

5. Discussion

The purpose of this study was to gain insights on the timeline of progression of osteoarticular TB in people from the Ligurian Neolithic period (Italy) through a novel approach, i.e. by evaluating its effects on body proportions and cross-sectional diaphyseal properties. Two skeletons in different ontogenetic phases were examined: the adolescent from Arene Candide (5), and the adult female from Arma dell'Aquila (1). When compared with the rest of the skeletal series, alterations in body proportions and/or significantly gracile diaphyses in those individuals would signal disturbances in development, long-term 'wasting', and a reduction of normal activity, which may be linked to the long-lasting incapacitating symptoms of TB, providing an independent assessment of disease progression.

5.1. Body size and proportions

Early disturbances in growth due to infectious disease and malnutrition may prevent the attainment of full stature potential (Robbins Schug, 2011; Vercellotti et al., 2014). Both AC5 and AQ1 display statures that are at the lower end of the range of variation for their age/sex class, but they do not appear to be severely stunted in growth. Moreover, body mass, as estimated from articular dimensions, is also low for both AC5 and AQ1, resulting in body proportions within normal variation, as described by the BMI. Although it is possible that the individuals at the bottom end of the stature distribution suffered from metabolic disturbances during growth, results for stature do not clearly evidence a major metabolic insult for the two individuals with ostearticular TB.

5.2. Postcranial mechanical rigidity

Diaphyseal cross-sectional size appears to be less genetically canalized compared to both articular size and length, and it is more sensitive to environmental factors (Ruff and Runestad, 1992; Ruff et al., 1993; Auerbach and Ruff, 2006; but see Lieberman et al., 2001). In addition, Z_p standardized by body size (or robusticity) is the relevant variable for making inferences on behavioral correlates (Ruff et al., 1993), or on the possible sub-ideal adaptation to the mechanical environment due to metabolic stress and lack of normal activity. AC5 is more gracile than his peers when considering lower limb rigidity (results are significant for the femur), although the comparative sample is small. This gracility is mainly due to a smaller total area of the section, while we could not find evidence of significant cortical thinning.

Most subperiosteal growth takes place during the pre- and peri-pubertal periods (Frisancho et al., 1970; Garn, 1970), and is determined by body size and activity-related mechanical loadings in the absence of significant metabolic disturbances (Lazenby, 1990; van der Muelen, 1997). Physical activity during adolescence is therefore one of the main determinants of diaphyseal structural properties scaled by body size (Ruff et al., 1994, 2006; Bass et al., 2002; Haapasalo, 1998; Haapasalo et al., 2000; Pearson and Lieberman, 2004). Clinical data and animal models show that, during development, reduced mechanical loadings (van der Muelen et al., 1995; Morey-Holton and Globus, 1998), compromised motor functions (Wren et al., 2011), or low levels of normal activity due to disease (hemophilia, Kovacs, 2008) result in smaller diaphyseal total areas. Murine models show that lack of activity during growth does arrest periosteal apposition, but does not result in significant endosteal expansion (van der Muelen et al., 1995; Morey-Holton and Globus, 1998). In addition, metabolic disturbances due to malnutrition disturb cross-sectional development, and there is evidence from both human and animal studies of a differential effect based on the timing, duration, and intensity of the metabolic insult. It appears that long-term chronic malnutrition results in smaller cross-sectional periosteal properties, with little effect on the medullary size (Himes et al., 1975; Glick and Rowe, 1981; Bozzini et al., 2013), while more severe insults lead to increased total area and especially medullary area, resulting in thinner cortices (Garn et al., 1969; DiVasta et al., 2007). Low robusticity in AC5 appears therefore more compatible with chronic malnutrition and a long-term period of inactivity at the critical age for bone modeling. In addition to the physical impairments due to Pott's spine, it is well known that TB leads to macro- and micronutrient malnutrition by interfering with protein and vitamin metabolism (Macallan, 1999; Schwenk and Macallan, 2000; van Lettow et al., 2004; del Rey et al., 2007; Wilbur et al., 2008). It is therefore reasonable to infer that the pattern of robusticity for AC5 is due to the slow progression of TB. However, more research is needed to understand how metabolic insults of a different nature, duration, and intensity affect skeletal development at various stages, and how the various skeletal traits interact under those circumstances (Robbins Schug and Goldman, 2014; Eleazer and Jankauskas, 2014). For example, in addition to an acute inflammatory state, TB leads to endocrine alterations which are not those expected during other severe alterations of homeostatic

balance; a drop in sex hormones but also a manifold increase in GH blood levels are evident (del Rey et al., 2007). Thus, modeling our expectations about the effects of TB on development following other metabolic disturbances may not be correct.

It is difficult to estimate the time span during which cross-sectional development may have been disturbed in AC5, given that his low standardized Z_p is influenced by both a lack of periosteal apposition and probable continued growth in length of the bones. However, the growth curve of non-standardized Z_p shows that values observed in AC5 can be attained by the age of 8-10 years for the lower limb, which could suggest metabolic disturbances for a few years before death, estimated at the age of 15 years (Formicola et al., 1987). Obviously, the presence of individual variation in mechanical competence makes this assessment very tentative, and using a larger sample of juvenile skeletons from the same site would have been advisable if it had existed. However, this timing correlates with the suggestion that AC5 suffered metabolic disturbances at an age of 11-12 years, based on the presence of Harris' lines of arrested growth and third molar enamel hypoplasia (Formicola et al., 1987).

Arene Candide 5 displays particularly gracility for the lower limb bones, while the humerus, albeit gracile, is well within the range of variation of the adolescent sample. There may be several factors contributing to this result. First, it may be due to the standardization method used: although Z_p in both the humerus and the femur is scaled by body mass × bone length, this method is more mechanically sound for weight-bearing skeletal elements (femur and tibia) than for the upper limb (Ruff, 2000; Ruff 2003a, 2003b), especially in juveniles (Cowgill, 2008). The values of standardized Z_p for the lower limb segments could therefore be a more accurate estimate of gracility levels for AC5. Second, differences in linear growth velocity between the humeri, the femur, and the tibia (Ruff 2003a, 2003b; Smith and Buschang, 2005) during the time when cross-sectional development was disturbed may have played a role. Finally, it is conceivable that the progressive destruction of the vertebral bodies and common complications of TB, such as weakness of the lower limbs and loss of muscular tone (Chandler and Page, 1940; Resnick and Niwayama, 1995; Turgut, 2001; Spiegel et al., 2005), had a greater impact on the ability to traverse the mountainous Ligurian landscape than on basic manipulative tasks, resulting in a differential level of inhibition of periosteal apposition.

The robusticity of AQ1 is within the range of variation of the adult Neolithic sample, suggesting that both her upper and lower limbs were adapted to being 'active' within her environment, and no cortical thinning is apparent. Following the completion of development, cortical thinning due to malnutrition, chronic disease, and inactivity (Garn et al., 1969; Himes et al., 1975; Hummert, 1983; Bass et al., 2002) is due to medullary area expansion, with little or no change in total area (e.g. Modlesky et al., 2005). Individuals with complete spinal cord injury lose bone mass inferior to the spinal damage, reaching a steady-state after 5-8 years in diaphyseal percent cortical area (Eser et al., 2004; Frotzel et al., 2008). Cortical bone loss at a steady-state is approximately 35% in the femur, and 25% in the tibia (Eser et al., 2004; Dudley-Javoroski and Shields, 2012). It can therefore be excluded that TB resulted in complete immobilization for several years. However, compared to immobilization due to spinal cord injuries, a decrease in physical activity in adulthood leads to more of a decrease in trabecular and bone mineral density than cortical area thickness or a change in mechanical properties (Tervo et al., 2009; Kontulainen et al., 2001; Nordström and Nordström, 2011; Voor et al., 2012). Therefore, results for AQ1 could be consistent with either a rapid course of the disease, or a period of metabolic disturbance and reduced activity, which could have endured for a few years before death. Bone remodeling in adults might not be sensitive and fast enough to be used to detect systemic changes due to TB in adults, especially in a bioarchaeological setting. Histological analyses, which are not possible at this stage of research, might be used to detect increased cortical porosity, and improve our understanding of disease progression (Klaus, 2014; Robbins Schug and Goldman, 2014; Eleazer and Jankauskas, 2016).

5.3. Cross-sectional indices and shape

Further insights on how TB impacted on activity may be provided by cross-sectional indices. Humeral asymmetry in AQ1 is very low, a characteristic that is typical of Ligurian Neolithic females, and might be due to the use of querns for cereal processing using both arms (Marchi et al., 2006; Sparacello et al., 2011). The upper limbs of AQ1 are therefore compatible, both in terms of robusticity and lateralization, with the profile of a normally active Ligurian Neolithic female. However, as seen above regarding robusticity, symmetry in AQ1 is compatible with both normal activity until close to death and therefore a rapid course of TB, and a disease

lasting a few years. In fact, symmetry may be the result of normal activity pattern established at an earlier age, which was maintained as both limbs responded equally to a longer-term lack of activity. Regarding AC5, his asymmetry is not suggestive of a specific behavioral pattern, being compatible with the level shown by non-active individuals (Trinkaus et al., 1994) and within the range of variation of the Neolithic sample. Therefore, in this setting, the study of asymmetry does not add to the evaluation of chronic versus acute metabolic insult.

Lower limb CSG shape indices are commonly associated with mobility levels (Carlson and Marchi, 2014), and Ligurian males show high values that are probably due to their pastoral economy (Marchi et al., 2006; 2011). During ontogeny, the orientation of femoral maximum bending rigidity shifts from medio-lateral (ML) to antero-posterior (AP) around 8-10 years of age, due to changes in body proportions and gait patterns (Cowgill, 2008; Cowgill et al., 2010; Gosman et al., 2013), and the femur acquires the adult pattern of anterolateral-posteromedial orientation in late adolescence (Goldman et al., 2009). Tibial shape at mid-shaft, which is roughly triangular in adulthood, progressively develops from a rounder cross section during growth due to an increase in diaphyseal rigidity on the AP plane (Gosman et al., 2013). As in adults, the degree of AP buttressing in late childhood and adolescence is presumably influenced by mobility levels (Cowgill, 2014). The results obtained for AC5 – particularly the high femoral shape index – are therefore compatible with the ontogenetic pattern of a highly mobile individual. However, this is not in contrast with the low femoral robusticity discussed above. It can be hypothesized that this individual was highly mobile in the landscape during late childhood/early adolescence, as dictated by early participation in subsistence-related activities within his pastoral society (Dyson-Hudson and Dyson-Hudson, 1980; Hobbs et al., 1999). Given that cross-sectional shape is retained even in individuals undergoing severe endosteal resorption due to paralysis (Gross and Rubin, 1995), it is possible that lower limb 'mobility indices' in AC5 were shaped as early as 8-10 years of age, before metabolic alterations disturbed cross-sectional development (see above). Adult females in the Ligurian Neolithic sample tend to show lower shape indices than males; the low values observed in AQ1 are therefore not uncommon and cannot be associated with a decrease in mobility due to skeletal TB.

Overall, the analysis of body size and cross-sectional geometric properties in AC5 and AQ1 depict a coherent scenario. The levels of cross-sectional robusticity and cortical area thickness in AQ1 tend to exclude the idea that the symptoms of TB prevented her from being part of subsistence practices for several years. On the other hand, the gracility of AC5, which is especially apparent in the femur, suggests a period of compromised periosteal apposition during ontogeny. The fact that femoral shape in this individual had shifted from a ML to AP orientation suggests that AC5 was normally active until a metabolic insult, possibly the onset of the active phase of TB, disrupted normal growth. Considering that the non-standardized values of upper and lower limb rigidity in AC5 are similar to those shown by younger individuals, this period of arrested periosteal apposition could have lasted a few years, suggesting a chronic condition. However, a larger comparative sample of juveniles would be needed to make this timeline estimate less speculative. Results, at least for the adolescent AC5, are not compatible with a rapid course of the disease, and appear more consistent with the time progression observed in untreated people with TB today (three years on average; Tiemersma et al., 2011). The analysis of postcranial properties therefore provides an independent estimate of disease progression which is in agreement with modern clinical data on the timing of the development of skeletal lesions. It appears that, by the Neolithic period, TB already had the potential to show the pattern of slow, chronic progression that is characteristic of diseases with a long history of host-pathogen coevolution, as suggested by recent genetic studies.

6. Conclusions

This study evaluated the course of skeletal TB in skeletons from the Italian Neolithic period using traits which are independent of osteoarticular lesions. We explored possible disturbances in growth (stature, body mass, and body mass index - BMI) and postcranial mechanical strength (via CSG) in two individuals from Liguria (northwestern Italy) who likely died with active tuberculous spondylitis: Arene Candide 5 and Arma dell'Aquila 1. The rationale of the analysis was that a rapid course of the disease would leave little systemic macroscopic change in the skeleton, except for the lesions directly caused by the TB pathogen, which could have been transmitted more rapidly in the past. Conversely, if TB, sickness, and lack of activity due to the symptomatic phase of TB endured for years, as often happens in untreated people

today, a clear signature of gracility and disruption of development should be apparent in the postcranium. Results, especially for the adolescent individual Arene Candide 5, suggest a period of compromised periosteal apposition in the diaphysis during ontogeny that could have lasted a few years. Thus, our findings are more consistent with the timeline of progression observed in untreated people with TB today. We therefore suggest that by the Neolithic period skeletal TB already showed the potential to displaying the pattern of chronic progression that is characteristic of diseases with long histories of host-pathogen co-evolution.

Acknowledgements

Grant sponsorship: Marie-Curie European Union COFUND/Durham Junior Research Fellowship [under EU grant agreement number 267209], Wolfson Institute for Health and Wellbeing Small Grant Scheme, and BABAO Small Grants 2015. We thank the Soprintendenza Archeologia della Liguria, the Museo di Archeologia Ligure (Genoa Pegli), the Civico Museo del Finale (Finale Ligure, SV), and the Museo di Storia Naturale dell'Università di Firenze (Florence) for permission to study the skeletal remains. The following people provided mentorship, advice, and help during data collection and analysis: Daniele Arobba, Paolo Biagi, Andrea De Pascale, Angiolo Del Lucchese, Irene Dori, Patrizia Garibaldi, Gwenaëlle Goude, Dalibor Ivezic, Katina Krasnec, Marcello Mannino, Liina Mansukoski, Irene Molinari, Jason Nadell, Chiara Panelli, Guido Rossi, Stefano Rossi, Elisabetta Starnini, Maria Tagliafico, Antonella Traverso, Giuseppe 'Cisque' Vicino, and Monica Zavattaro. We are particularly grateful to Vincenzo Formicola for providing original radiographs and continuous support during the study. Thanks to the Editor of this Journal and to two anonymous reviewers for providing useful comments that improved the quality of the paper.

Literature Cited

Arobba D, Biagi P, Formicola V, Isetti E, Nisbet R. 1987. Nuove osservazioni sull'Arma dell'Aquila (Finale Ligure – Savona). In: Parenti R, editor. Atti della XXVI Riunione Scientifica, il Neolitico in Italia, Firenze 7-10 Novembre 1985. Firenze: Istituto Italiano di Preistoria e Protostoria. p 541-551.

Auerbach RM, Ruff CB. 2006. Limb bone bilateral asymmetry: variability and commonality among modern humans. J Hum Evol 50:203-218.

Aufderheide A, Rodríguez Martin C. 1998. The Cambridge encyclopedia of human paleopathology. Cambridge: Cambridge University Press.

Bass SL, Saxon L, Daly RM, Turner CH, and Robling AG, Seeman E, Stuckey S. 2002. The effect of mechanical loading on the size and shape of bone in pre-, peri, and postpubertal girls: a study in tennis players. J Bone Miner Res 17:2274–2280.

Benvenuti C. 2012. Revisione dei materiali scheletrici provenienti dalla caverna delle Arene Candide (SV) presenti nella Sezione di Antropologia ed Etnologia del Museo di Storia Naturale. MA Thesis, Università di Firenze.

Bos, K.I., Harkins, K.M., Herbig, A., Coscolla, M., Weber, N., Comas, I., Forrest, S.A., Bryant, J.M., Harris, S.R., Schuenemann, V.J., Campbell, T.J., Majander, K., Wilbur, A.K., Guichon, R.A., Wolfe Steadman, D.L., Cook, D.C., Niemann, S., Behr, M.A., Zumarraga, M., Bastida, R., Huson, D., Nieselt, K., Young, D., Parkhill, J., Buikstra, J.E., Gagneux, S., Stone, A.C., Krause, J. 2014. Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis. Nat. 514, 494–497.

Bozzini C, Picasso EO, Champin GM, Alippi RM, Bozzini CE. 2013. Mechanical testing at whole-bone level of the femur in immature rats stunted by cornstarch consumption. Food Funct 4:1453.

Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, Garnier T, Gutierrez C, Hewinson G, Kremer K, Parsons LM, Pym AS, Samper S, van Soolingen D, Cole ST. 2002. A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proc Natl Acad Sci USA 99:3684-3689.

Bruzek J. 2002. A method for visual determination of sex using the human hip bone. Am J Phys Anthrop 117:157–168.

Buikstra, JE and Ubelaker, DH, editors. 1994. Standards for Data Collection from Human Skeletal Remains. Fayetteville: Arkansas Archaeological Survey Research Series No 44.

Canci A, Minozzi S, Borgognini Tarli S. 1996. New evidence of tuberculous spondylitis from Neolithic Liguria (Italy). Int J Osteoarch 6:497-501.

Carlson K, Marchi D, editors. 2014. Reconstructing mobility: environmental, behavioral, and morphological determinants. New York: Springer.

Chandler FA Page MA. 1940. Tuberculosis of the spine. J Bone Joint Surg 22: 851–859.

Comas I, Coscolla M, Luo T, Borrell S, Holt K, E, Kato-Maeda M, Parkhill J, Malla B, Berg S, Thwaites G, Yeboah-Manu D, Bothamley G, Mei J, Wei L, Bentley S, Harris S, R, Niemann S, Diel R, Aseffa A, Gao Q, Young D, Gagneux S. 2013. Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans. Nat Gen 45:1176-1184.

Cowgill LW. 2008. The ontogeny of recent and late Pleistocene human postcranial robusticity. Ph. D. Dissertation, Washington University, St Louis.

Cowgill LW. 2010. The ontogeny of Holocene and Late Pleistocene human postcranial strength. Am J Phys Anthropol 141:16-37.

Cowgill LW. 2014. Femoral diaphyseal shape and mobility: an ontogenetic perspective. In: Carlson K, Marchi D, editors. Reconstructing mobility: environmental, behavioral, and morphological determinants. New York: Springer. p 193-208.

Cowgill LW, Warrener A, Pontzer H, Ocobock C. 2010. Waddling and toddling: the biomechanical effects of an immature gait. Am J Phys Anthropol 143:52-61.

Davies T, Shaw CN, Stock JT. 2012. A test of a new method and software for the rapid estimation of cross-sectional geometric properties of long bone diaphyses from 3D laser surface scans. Archaeol Anthropol Sci 4:277–290.

De Pascale A. 2008. Le prime esplorazioni nelle caverne ossifere del Finalese: tracce, ipotesi e scoperte ad opera di Issel, Perrando, Morelli, Rovereto, Rossi, Amerano... In: De Pascale A, Del Lucchese A, Raggio O, editors. La nascita della Paletnologia in Liguria: personaggi, scoperte e collezioni tra XIX e XX secolo', Atti del Convegno (Finale Ligure Borgo, 22-23 settembre 2006). Bordighera: Istituto Internazionale di Studi Liguri. p 233-248.

Del Lucchese A. 1997. The Neolithic burials from Arene Candide cave – the Bernabò Brea-Cardini excavations. n: Maggi R, editor. Arene Candide: a functional and environmental assessment of the Holocene sequence (excavations Bernabò Brea-Cardini 1940–50). Mem Ist Ital Paleontol Um 5:605-611.

Del Lucchese A. 2014. Nuovi dati sul pieno Neolitico del Finalese dal Riparo di Pian del Ciliegio. In: Massabò B, editor. Atti del Convegno 'Il Pieno Neolitico in Italia', Finale Ligure (8 – 10 Giugno 2009). Bordighera: Istituto Internazionale di Studi Liguri. p. 405-413.

Del Rey A, Mahuad CV, Bozza V, Bogue C, Farroni MA, Bay ML, Bottasso OA, Besedovsky. 2007. Endocrine and cytokine responses in humans with pulmonary tuberculosis. Brain Behav Immun 21:171-179.

Di Vasta AD, Beck TJ, Petit MA, Feldman HA, Le Boff MS, Gordon CM. 2007. Bone cross-sectional geometry in adolescents and young women with anorexia nervosa: a hip structural analysis study. Osteoporos Int 18:797-804.

Dudley-Javoroski S, Shields RK. 2012. Regional cortical and trabecular bone loss after spinal cord injury. J Rehabil Res Dev 49(9):1365-1376

Dyson-Hudson R, Dyson-Hudson N. 1980. Nomadic pastoralism. Ann Rev Anthropol 9:15-61.

Eleazer CD, Jankauskas R. 2016 (In Press). Mechanical and metabolic interactions in cortical bone development. Am J Phys Anthropol DOI: 10.1002/ajpa.22967.

El-Najjar MY. 1981. Skeletal changes in tuberculosis: the Hamann-Todd collection. In: Buikstra JE, editor. Prehistoric tuberculosis in the Americas. Evanston: Northwestern University Archaeological Program. p 85-97.

Eser P, Frotzler A, Zehnder Y, Wick L, Knecht H, Denoth J, Schiessl H. 2004. Relationship between the duration of paralysis and bone structure: a pQCT study of spinal cord injured individuals. Bone 34:869-80.

Formicola V, Franceschi M. 1996. Regression equations for estimating stature from long bones of Early Holocene European samples. Am J Phys Anthropol 100:83–88.

Formicola V, Milanesi Q, Scarsini C. 1987. Evidence of spinal tuberculosis at the beginning of the fourth millennium BC from Arene Candide cave (Liguria, Italy). Am J Phys Anthropol 72:1-6.

Fox JG, Campbell LH, Snyder SB, Reed C, Soave OA. 1974. Tuberculous spondylitis and Pott's paraplegia in a rhesus monkey (Macaca mulatta). Lab Anim Sci 24:335-339.

Frisancho RA, Garn SM, Ascoli W. 1970. Subpreiosteal and endosteal bone apposition during adolescence. Hum Biol 42:639-664.

Frotzel A, Berger M, Knecht H, Eser P. 2008. Bone steady-state is established at reduced bone strength after spinal cord injury: a longitudinal study using peripheral quantitative computed tomography (pQCT). Bone 43:549-555.

Garg RK, Somvanshi DS. 2011. Spinal tuberculosis: A review. J Spinal Cord Med 34(5): 440-454.

Garn SM. 1970. The earlier gain and the later loss of cortical bone. Springfield, IL: Charles C. Thomas.

Garn SM, Rohmann CB, Behar M, Viteri F, Guzman MA. 1964. Compact bone deficiency in protein-calorie malnutrition. Science 145: 1444-1445.

Garn SM, Guzman MA, Wagner B. 1969. Subperiosteal gain and endosteal loss in protein-calorie malnutrition. Am J Phys Anthropol 30:153–155.

Gill WP, Harik NS, Whiddon MR, Liao RP, Mittler JE, Sherman DR. 2009. A replication clock for Mycobacterium tuberculosis. Nat Med 15(2): 211–214.

Girling DJ, Darbyshire JH, Humphries MJ, O'Mahoney, SG. Extra-pulmunary tuberculosis. Brit Med Bull 44:738-756.

Glick PL, Rowe DJ. 1981. Effects of chronic protein deficiency on skeletal development of young rats. Calcif Tissue Int 33:223-231.

Goldman HM, McFarlin SC, Cooper DML, Thomas CDL, Clement JG. 2009. Ontogenetic patterning of cortical bone microstructure and geometry at the human mid-shaft femur. Anat Rec 292:48-64.

Gosman, J. H. Hubbell, Z. R. Shaw, C. N. Ryan, T. M. 2013. Development of cortical bone geometry in the human femoral and tibial diaphysis. Anat Rec 296: 774-787.

Gross TS, Rubin CT. 1995. Uniformity of resorptive bone loss induced by disuse. J Orthop Res 13:708-714.

Haapasalo H. 1998. Physical activity and growing bone: development of peak bone mass with special reference to the effects of unilateral activity. Ann Chir Gynaecol 87:250–252.

Haapasalo H, Kontulainen S, Sievänen H, Kannus P, Järvinen M, Vuori I. 2000. Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone

density: a peripheral quantitative computed tomography study of the upper arms of male tennis players. Bone 27:351–357.

Haavikko K. 1970. The formation and the alveolar and clinical eruption of the permanent teeth. An orthopantographic study. Proc Finn Dent Soc 66: 101–170.

Hershberg, R., Lipatov, M., Small, P.M., Sheffer, H., Niemann, S., Homolka, S., Roach, J.C., Kremer, K., Petrov, D.A., Feldman, M.W., Gagneux, S. 2008. High Functional Diversity in Mycobacterium tuberculosis Driven by Genetic Drift and Human Demography. PLoS Biol. 6(12), e311.

Hobbs S, McKechnie J, Lavalette M. 1999. Child labor: a world history companion. Santa Barbara: ABC-CLIO.

Himes JH, Martorell R, Habicht JP, Yarbrough C, Malina RM, Klein RE. 1975. Patterns of cortical bone growth in moderately malnourished preschool children. Hum Biol 47:337–350.

Hirsh AE, Tsolaki AG, DeRiemer K, Feldman MW, Small PM. 2004. Stable association between strains of Mycobacterium tuberculosis and their human host populations. Proc Natl Acad Sci USA 101: 4871–4876.

Isaza R. 2003. Tuberculosis in all taxa. In: Fowler ME, Miller RE, editors. Zoo and wild 20 animal medicine. Pennsylvania: WB Saunders. p 689-696.

Klaus HD. 2014. Frontiers in the bioarchaeology of stress and disease: cross-disciplinary perspectives from pathophysiology, human biology, and epidemiology. Am J Phys Anthropol 155:294-308.

Kontulainen S, Kannus P, Haapasalo H, Sievanen H, Pasanen M, Heinonen A, Oja P, Vuori I. 2001. Good maintenance of exercise-induced bone gain with decreased training of female tennis and squash players: a prospective 5-year follow-up study of young and old starters and controls. J Bone Min Res 169:195-201.

Kovacs CS. 2008. Hemophilia, low bone mass, and osteopenia/osteoporosis. Transfus Apher Sci 38:33-40.

Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, Wei R, Curtin LR, Roche AF, Johnson CL. 2002. CDC growth charts for the United States: Methods and development. National Center for Health Statistics. Vital Health Stat 11(246).

Lazenby RA. 1990. Continuing periosteal apposition II: the significance of peak bone mass, strain equilibrium, and age related activity differentials for mechanical compensation in human tubular bones. Am J Phys Anthropol 82:473–484.

Le Bras-Goude G, Binder D, Formicola V, Duday H, Couture-Veschambre C, Hublin J-J, Richards MP. 2006, Stratégies de subsistance et analyse culturelle de populations néolithiques de Ligurie: approche par l'étude isotopique ($\delta^{13}C$ et $\delta^{15}N$) des restes osseux. Bull Mem Soc Anthropol Paris 18:45-55.

Lieberman, D.E., Devlin, M.J., Pearson, O.M., 2001. Articular area response to mechanical loading: effects of exercise, age, and skeletal location. Am J Phys Anthropol 116:266-277.

Luk KDK. 1999. Tuberculosis of the spine in the new millennium. Europ J Spine 8: 338–345.

Macallan DC. 1999. Malnutrition in tuberculosis. Diagn Microbiol Infect Dis 34:153-157.

Macintosh AA, Davies TG, Ryan TM, Shaw CN, Stock JT. 2013. Periosteal versus true Cross-Sectional Geometry: a comparison along humeral, femoral, and tibial diaphysis. Am J Phys Anthropol 150:442–452.

Macintosh AA, Pinhasi R, Stock JT. 2014. Lower limb skeletal biomechanics track long-term decline in mobility across ~ 6150 years of agriculture in Central Europe. J Archaeol Sci 52:1-15.

Maggi R. 1997a. The radiocarbon chronology. In: Maggi R, editor. Arene Candide: a functional and environmental assessment of the Holocene sequence (excavations Bernabò Brea-Cardini 1940–50). Mem Ist Ital Paleont Um 5:31–52.

Maggi R. 1997b. Summary: a modern excavation carried out fifty years ago. In: Maggi R, editor. Arene Candide: a functional and environmental assessment of the Holocene sequence (excavations Bernabò Brea-Cardini 1940–50). Mem Ist Ital Paleontol Um 5:635–642.

Maggi R, Nisbet R. 1991. Prehistoric pastoralism in Liguria. In: Maggi R, Nisbet R, Barker G, editors. Archeologia della Pastorizia nell'Europa Meridionale. Riv St Lig 56:265-296.

Marchi D. 2008. Relationships between limb cross-sectional geometry and mobility: the case of a Neolithic sample from Italy. Am J Phys Anthropol 137:188-200.

Marchi D, Sparacello VS, Holt BM, Formicola V. 2006. Biomechanical approach to the reconstruction of activity patterns in Neolithic Western Liguria, Italy. Am J Phys Anthropol 131:447–455.

Marchi D, Sparacello VS, Shaw CN. 2011. Mobility and lower limb robusticity of a pastoralist Neolithic population from North-Western Italy. In: Pinhasi R, Stock J, editors. Human bioarchaeology of the Transition to Agriculture. New York: Wiley-Liss. p 317–346.

Martin JE, Cole WC, Whitney RA Jr. 1968. Tuberculosis of the spine (Pott's Disease) in a rhesus monkey (Macaca mulatta). J Am Vet Med Assoc 153:914-917.

Mays S, Ives R, Brickley M. 2009. The effects of socioeconomic status on endochondral and appositional bone growth, and acquisition of cortical bone in children from 19th century Birmingham. England. Am J Phys Anthropol 140:410–416.

Meghji S, White PA, Reddi K, Heron K, Henderson B, Zaliani A, Fossati G, Mascagni P, Hunt JF, Roberts MM, Coates ARM. 1997. Mycobacterium tuberculosis Chaperonin 10 Stimulates Bone Resorption: A Potential Contributory Factor in Pott's Disease. J Exp Med 186(8): 1241-1246.

Minitab Inc. 2010. Minitab 16. State College, Minitab Inc.

Modlesky CM, Slade JM, Bickel CS, Meyer RA, Dudley GA. 2005. Deteriorated geometric structure and strength of the midfemur in men with complete spinal cord injury. Bone 36:331-339.

Morey-Holton ER, Globus RK. 1998. Hindlimb unloading of growing rats: a model for predicting skeletal changes during space flight. Bone 22(5):83-88.

Munoz-Elias EJ, Timm J, Botha T, Chan WT, Gomez JE, McKinney JD. 2005. Replication dynamics of Mycobacterium tuberculosis in chronically infected mice. Infect Immun 73:546-551.

Nagurka ML, Hayes WC. 1980. An interactive graphics package for calculating cross-sectional properties of complex shapes. J Biomech 13:59–64.

Nordström A, Nordström P. 2011. The effect of detraining on bone. Open Bone J 3:22-30.

O'Neill MC, Ruff CB. 2004. Estimating human long bone cross-sectional geometric properties: a comparison of noninvasive methods. J Hum Evol 47:221–235.

Pagani S. 2012. Revisione dei resti scheletrici umani provenienti dalla Grotta Pollera (SV) presenti nel Museo di Storia Naturale dell'Università di Firenze. BA Honors Thesis, Università di Pavia.

Palkovich AM. 1981. Tuberculosis epidemiology in two Arikara skeletal samples: a study of disease impact. In: Buikstra JE, editor. Prehistoric tuberculosis in the Americas. Evanston: Northwestern University Archaeological Program. p 161-175.

Palmer RA, Strobeck C. 1992. Fluctuating asymmetry as a measure of developmental stability: implications of non-normal distributions and power of statistical tests. Acta Zool Fennica 191:57-72.

Parenti R, Messeri P. 1962. I resti scheletrici umani del Neolitico Ligure. Palaeontogr Ital 50:5-165.

Pearson OM, Cordero RM, Busby A M. 2006. How different were Neanderthals' habitual activities? A comparative analysis with diverse groups of recent humans. In: K Harvati, T Harrison, editors. Neanderthals Revisited: New Approaches and Perspectives. New York: Springer.

Pearson OM, Lieberman DE. 2004. The aging of Wolff ’s ‘Law’:ontogeny and response to mechanical loading in cortical bone. Am J Phys Anthropol 47:63–99.

Pearson OM, Sparacello VS. In Press. Behavioral differences between near eastern Neandertals and early modern humans from Skhul and Qafzeh: an assessment based on comparative samples of Holocene humans. In: Hovers E, Marom A, editors. Human Paleontology and Prehistory: Contributions in Honor of Yoel Rak. New York:Springer.

Peña JC, Ho W-Z. 2015. Monkey models of tuberculosis: lessons learned. Infect Immun 83:852–862.

Resnick D, Niwayama G. 1995. Osteomyelitis, septic arthritis, and soft tissue infection: Organisms. In: Resnick D, editor. Diagnosis of bone and joint disorders. Edinburgh: W. B. Saunders. p. 2448–2558.

Rhodes JA, Knüsel CJ. 2005. Activity-related skeletal change in medieval humeri: cross-sectional and architectural alterations. Am J Phys Anthropol 128:536–546.

Robbins Schug G. 2011. Bioarchaeology and climate change. A view from South Asian Prehistory. Gainesville: University Press of Florida.

Robbins Schug G, Goldman HM. 2014. Birth is but our death begun: a bioarchaeological assessment of skeletal emaciation in immature human skeletons in the context of environmental, social, and subsistence transition. Am J Phys Anthropol 155:243-259.

Robbins Schug G, Gupta S, Cowgill LW, Sciulli PW, Blatt SH. 2013. Panel regression formulae for estimating stature and body mass from immature human skeletons: a statistical approach without reference to specific age estimates. J Archaeol Sci 40:1-11.

Roberts CA, Buikstra JE. 2003. The bioarchaeology of tuberculosis. Gainesville: University Press of Florida.

Roberts CA, 2015. Old World tuberculosis: evidence from human remains with a review of current research and future prospects. Tuberculosis 95: S117-S121.

Rowley-Conwy P. 1997. The animal bones from Arene Candide Holocene sequence: final report. In: Maggi R, editor. Arene Candide: A Functional and Environmental Assessment of the Holocene Sequence Excavations Bernabò Brea-Cardini 1940–1950. Mem Ist It Paleont Um 5:153–195.

Ruff CB. 1995. Biomechanics of the hip and birth in early Homo. Am J Phys Anthropol 98:527–574.

Ruff CB. 2000. Body size, body shape, and long bone strength in modern humans. J Hum Evol 38:269–290.

Ruff CB. 2003a. Growth in bone strength, body size, and muscle size in a juvenile longitudinal sample. Bone 33:317-329.

Ruff CB. 2003b. Ontogenetic adaptations to bipedalism: age changes in femoral to humeral length and strength proportions in humans, with a comparison to baboons. J Hum Evol 45:317-349.

Ruff CB. 2007. Body size prediction from juvenile skeletal remains. Am J Phys Anthropol 133:698–716.

Ruff CB. 2010. Structural analyses of postcranial remains. In: Morgan ME, editor. Pecos Pueblo revisited: the biological and social context. Cambridge, MA: Papers of the Peabody Museum of Archeology and Ethnology, Harvard University.

Ruff CB, Garofalo E, Holmes MA. 2013. Interpreting skeletal growth in the past from a functional and physiological perspective. Am J Phys Anthropol 150: 29-37.

Ruff CB, Walker A, Trinkaus E. 1994. Postcranial robusticity in Homo, III: ontogeny. Am J Phys Anthropol 93:35-54.

Ruff CB, Holt B, Trinkaus E. 2006. Who's afraid of the big bad Wolff? 'Wolff 's law' and bone functional adaptation. Am J Phys Anthropol 129:484–498.

Ruff CB, Runestad J. 1992. Primate limb bone structural adaptations. Ann Rev Anthropol 21:407–433.

Ruff CB, Trinkaus E, Walker A, Larsen CS. 1993. Postcranial robusticity in Homo. I: temporal trends and mechanical interpretation. Am J Phys Anthropol 91:21–54.

Sapolsky RM, Else JG. 1987. Bovine tuberculosis in a wild baboon population: epidemiological aspects. J Med Primatol 16:229-235.

Scheuer L, Black S. 2004. The juvenile skeleton. New York: Academic Press.

Schluger NW, Rom WN. 1998. The host immune response to tuberculosis. Am J Respir Crit Care Med 157:679-691.

Schutkowski H. 1993. Sex determination of infant and juvenile skeletons: I. Morphognostic features. Am J Phys Anthropol 90:199-205.

Schwenk A, Macallan DC. 2000. Tuberculosis, malnutrition and wasting. Curr Opin Clin Nutr Metab Care 3:285-291.

Smith SL, Buschang PH. 2005. Longitudinal models of long bone growth during adolescence. Am J Hum Biol 17:731-745.

Sparacello VS, Marchi D. 2008. Mobility and subsistence economy: a diachronic comparison between two groups settled in the same geographical area (Liguria, Italy). Am J Phys Anthropol 136:485-495.

Sparacello VS, Marchi D, Shaw CS. 2014. The importance of considering fibular robusticity when inferring the mobility patterns of past populations In: Carlson K, Marchi D, editors.

Reconstructing mobility: environmental, behavioral, and morphological determinants. New York: Springer. p. 91-111.

Sparacello VS, Pearson OM. 2010. The importance of accounting for the area of the medullary cavity in cross-sectional geometry: a test based on the femoral midshaft. Am J Phys Anthropol 143:612-624.

Sparacello VS, Pearson OM, Coppa A, Marchi D. 2011. Changes in robusticity in an Iron Age agropastoral group: the Samnites from the Alfedena necropolis (Abruzzo, Central Italy). Am J Phys Anthropol 144:119–130.

Spiegel DA, Singh GK, Banskota AK. 2005. Tuberculosis of the musculoskeletal system. Tech Orthopaed 20:167-178.

Statsoft. 2011. Statistica. Tulsa: Statsoft.

Stock JT, Shaw CN. 2007. Which measures of skeletal robusticity are robust? A comparison of external methods of quantifying diaphyseal strength to cross-sectional geometric properties. Am J Phys Anthropol 134:412–423.

Temple DH, Goodman AH. 2014. Bioarchaeology has a 'Health' problem: conceptualizing 'stress' and 'health' in bioarchaeological research. Am J Phys Anthropol 155:186-191.

Tervo T, Nordstrom P, Neovius M, Nordstrom A. 2009. Reduced physical activity corresponds with greater bone loss at the trabecular than the cortical bone sites in men. Bone: 45(6):1073-1078.

Tiemersma EW, van der Werf MJ, Borgdorff MW, Williams BG, Nagelkerke NJD (2011) Natural History of Tuberculosis: Duration and Fatality of Untreated Pulmonary Tuberculosis in HIV Negative Patients: A Systematic Review. PLoS ONE 6(4): e17601.

Trinkaus E, Churchill SE, Ruff CB. 1994. Postcranial robusticity in Homo. II. humeral bilateral asymmetry and bone plasticity. Am J Phys Anthropol 93:1–34.

Trinkaus E, Ruff CB. 1999. Diaphyseal cross-sectional geometry of Near Eastern Middle Paleolithic humans: the femur. J Archaeol Sci 26:409–424.

Trinkaus E, Ruff CB. 2012. Femoral and tibial diaphyseal cross-sectional geometry in Pleistocene Homo. Paleoanth 2012:13-62.

Tuli SM. 2013. Historical aspects of Pott's disease (spinal tuberculosis) management. Eur Spine J 22:529–S538.

Turgut M. 2001. Spinal tuberculosis (Pott's disease): its clinical presentation, surgical management, and outcome. A survey study on 694 patients. Neurosurg Rev 24:8-13.

Ubelaker DH. 1979. Human Skeletal Remains: Excavation, Analysis and Interpretation. Washington, DC: Smithsonian Institute Press.

Ulrichs T, Kosmiadi GA, Jorg S, Pradl L, Titukhina M, Mishenko V, Gushina N, Kaufmann SHE. 2005. Differential organization of the local immune response in patients with active cavitary 10 tuberculosis or with nonprogressive tuberculoma. J Infect Dis 192:89-97.

van der Muelen MCH. 1997. Diaphyseal bone growth and adaptation: models and data. In: Lowet G, Rüegsegger P, Weinans H, Meunier A. Boner research in biomechanics. Clifton: IOS Press. p 17-23.

van der Meulen MCH, Morey-Holton ER, Carter DR. 1995. Hindlimb suspension diminishes femoral cross-sectional growth in the rat. J Orthopaed Res 13:700 –707.

Van Lettow M, Kumwenda JJ, Harries AD, Whalen CC, Taha TE, Kumwenda N, Kang'ombe C, Semba RD. 2004. Malnutrition and the severity of lung disease in adults with pulmonary tuberculosis in Malawi. Int J Tuberc Lung Dis 8:211-7.

Vercellotti G, Piperata BA, Agnew AM, Wilson WM, Dufour DL, Reina JC, Boano R, Justus HM, Larsen CS, Stout SD, Sciulli PW. 2014. Exploring the multidimensionality of stature variation in the past through comparisons of archaeological and living populations. Am J Phys Anthropol 155:229-242.

Vigorita VJ. 2008. Orthopaedic pathology. Philadelphia: Lippincott Williams & Wilkins.

Voor MJ, Brown EH, Xu Q, Waddell SW, Burden RL, Jr, Burke DA, Magnuson DS. 2012. Bone loss following spinal cord injury in a rat model. J Neurotrauma 29:1676-82.

Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. 1997. Predicting obesity in young adulthood from childhood and parental obesity. New Engl J Med 37:869–873.

Wilbur A K, Farnbach AW, Knudson KJ, Buikstra JE. 2008. Diet, Tuberculosis, and the Paleopathological Record. Curr Anthropol 49:963–991.

Wirth, T., Hildebrand, F., Allix-Béguec, C., Wölbeling, F., Kubica, T., Kremer, K., van Soolingen, D., Rüsch-Gerdes, S., Locht, C., Brisse, S., Meyer, A., Supply, P., Niemann, S. 2008. Origin, Spread and Demography of the Mycobacterium tuberculosis Complex. PLoS Pathog 4(9), e1000160.

Wood JW, Milner GR, Harpending HC, Weiss KM. 1992. The osteological paradox. Problems of inferring health from skeletal samples. Current Anthropology 33:343-370.

Wren TAL, Lee DC, Kay RM, Dorey FJ, Gilsanz V. 2011. Bone density and size in ambulatory children with cerebral palsy. Dev Med Child Neurol 53:137-141.

Figure Legends

Figure 1 – Geographic collocation of Liguria, and distribution of the Neolithic sites from which skeletal remains included in this research have been unearthed. 1: Arene Candide; 2: Pollera; 3 Arma dell'Aquila; 4: Boragni; 5; Pian del Ciliegio; 6: Bergeggi. Created with Google Maps™ and d-maps.com.

Figure 2 – Individual value plots of stature (a) and body mass (b) against age categories. The individuals showing skeletal signs of tuberculosis are the labeled triangles (AC5: Arene Candide 5; AQ1: Arma dell'Aquila 1).

Figure 3 – Individual value plot of body mass index (BMI: body mass/ stature^2) against age categories. The individuals showing skeletal signs of tuberculosis are the labeled triangles (AC5: Arene Candide 5; AQ1: Arma dell'Aquila 1).

Figure 4 – Individual value plots of humeral (a: right; b: left), femoral (c), and tibial (d) non-standardized bone rigidity (Z_p: $J^{0.73}$) against age categories. The individuals showing skeletal signs of tuberculosis are the labeled triangles (AC5: Arene Candide 5; AQ1: Arma dell'Aquila 1).

Figure 5 – Individual value plots of humeral (a: right; b: left), femoral (c), and tibial (d) bone rigidity (Z_p: $J^{0.73}$) scaled by body size (body mass × bone length) against age categories. The

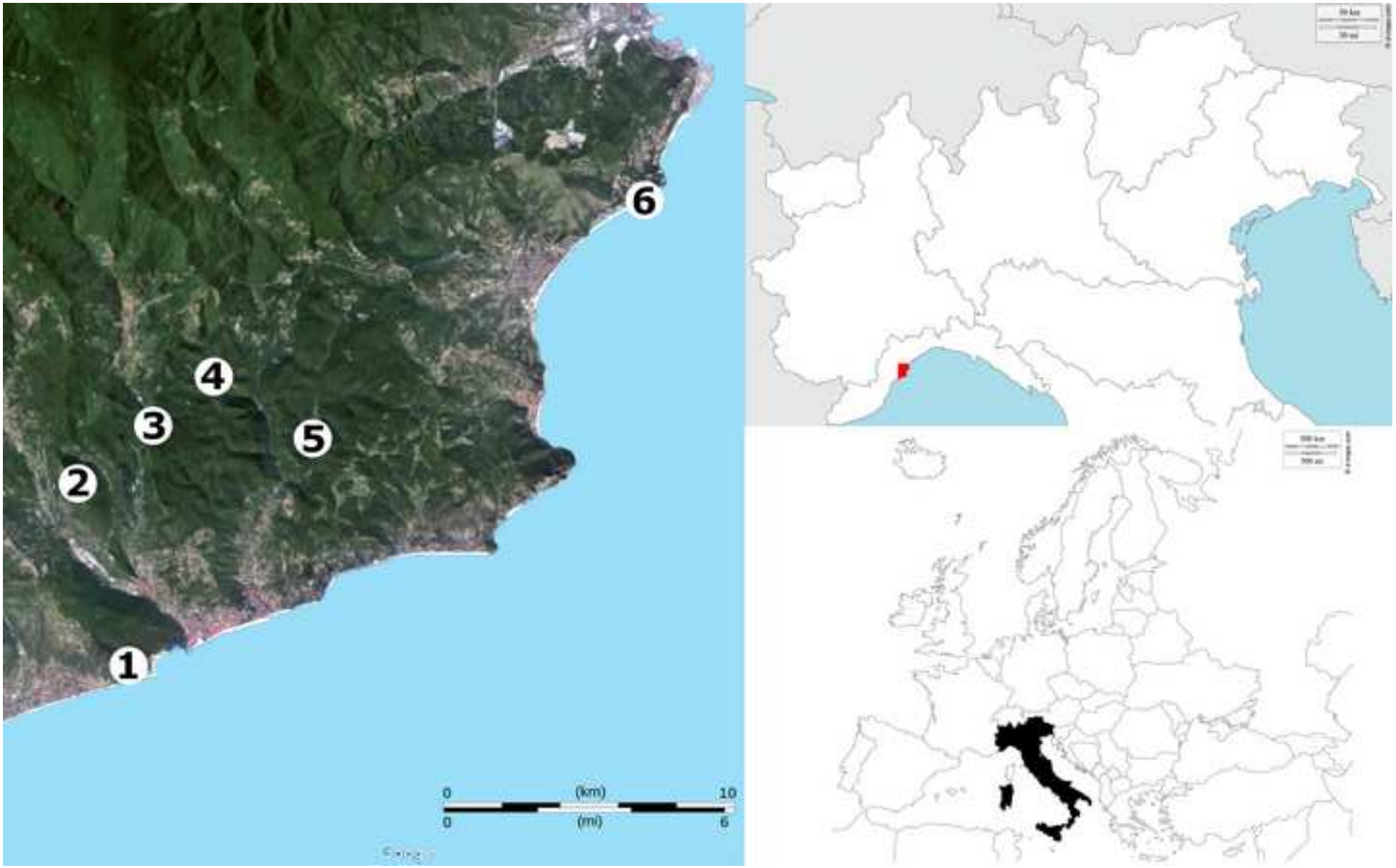
individuals showing skeletal signs of tuberculosis are the labeled triangles (AC5: Arene Candide 5; AQ1: Arma dell'Aquila 1).

Figure 6 – Individual value plots of the femoral shape index I_x/I_y (a) and the tibial shape index I_{max}/I_{min} (b) against age categories. The individuals showing skeletal signs of tuberculosis are the labeled triangles (AC5: Arene Candide 5; AQ1: Arma dell'Aquila 1).

Figure 8 – Scatterplots of medial and lateral tibial cortical thickness against tibial diameter (a), and of cortical area against total area for the humeri (b: right; c: left), and femur (d). The line represents the least square linear fit of the data. The individuals showing skeletal signs of tuberculosis are the labeled triangles (AC5: Arene Candide 5; AQ1: Arma dell'Aquila 1).

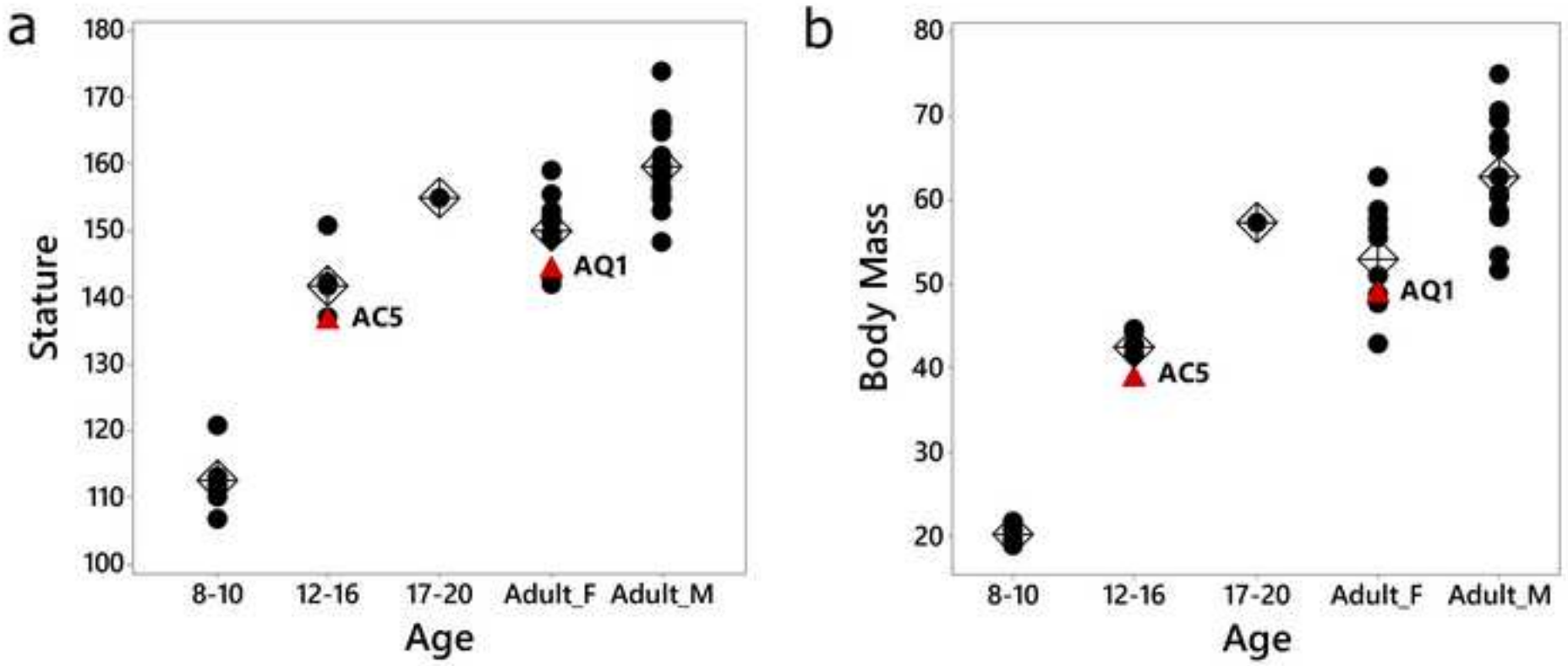
Figure

Click here to download high resolution image



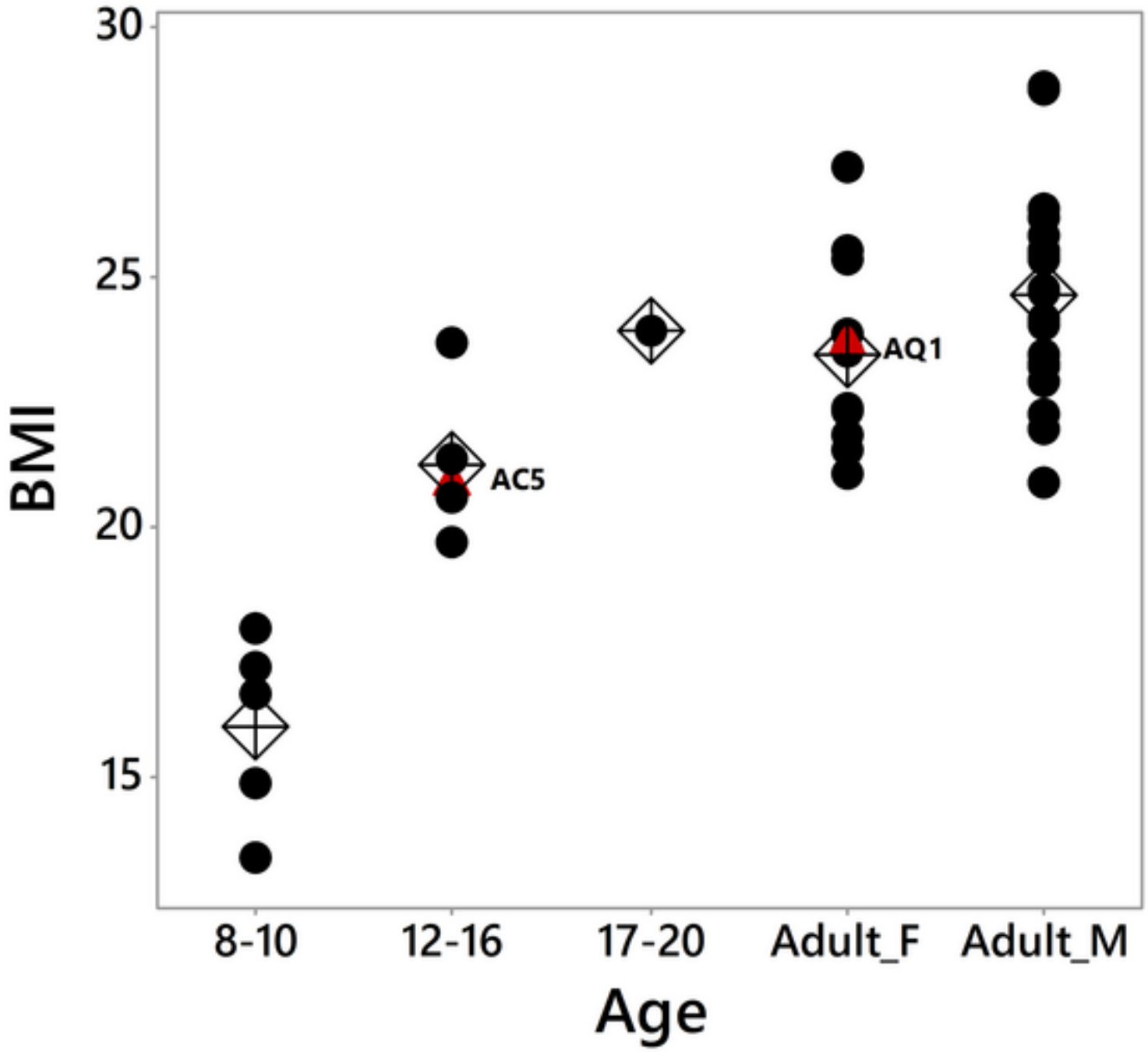
Figure

Click here to download high resolution image



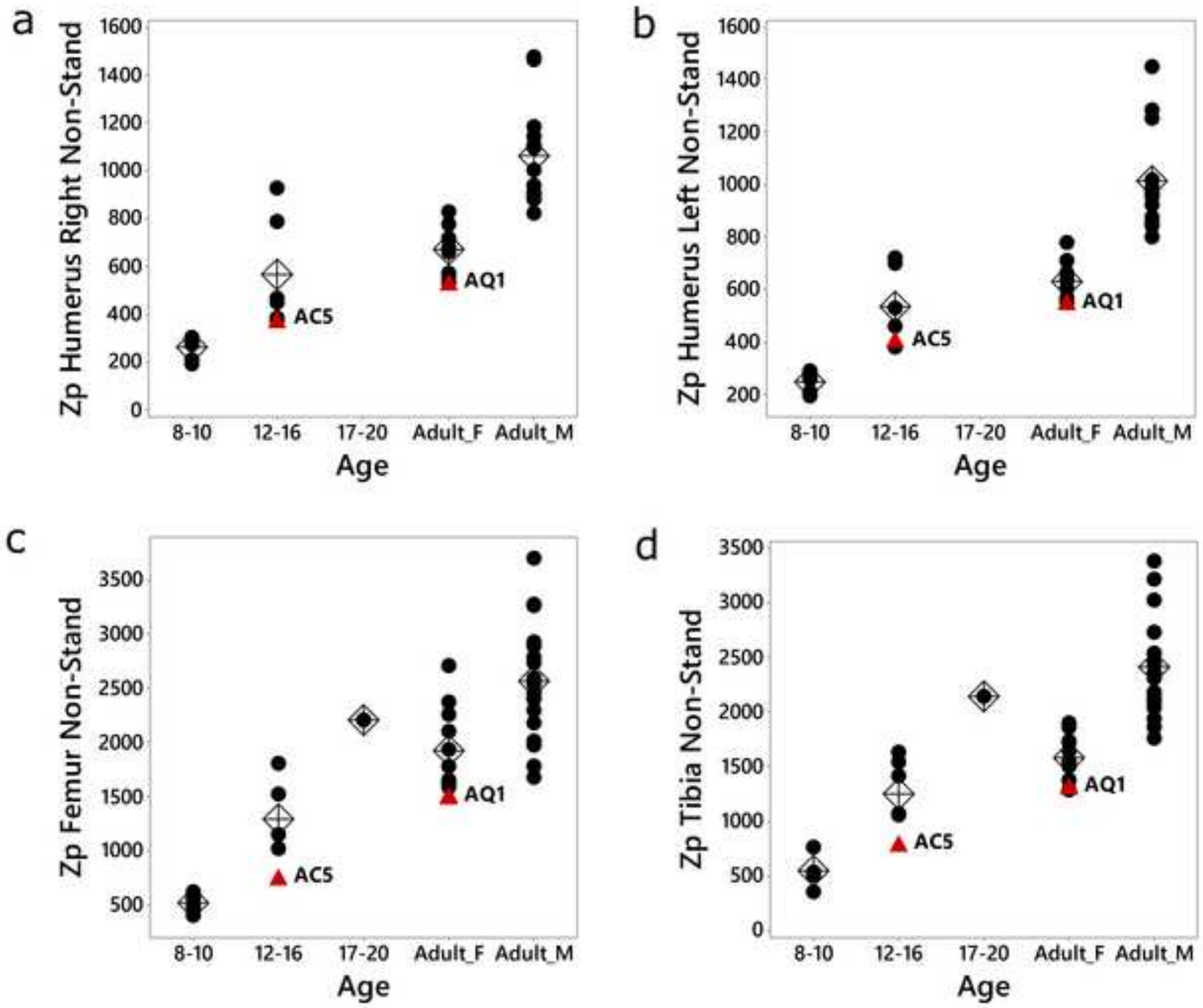
Figure

Click here to download high resolution image



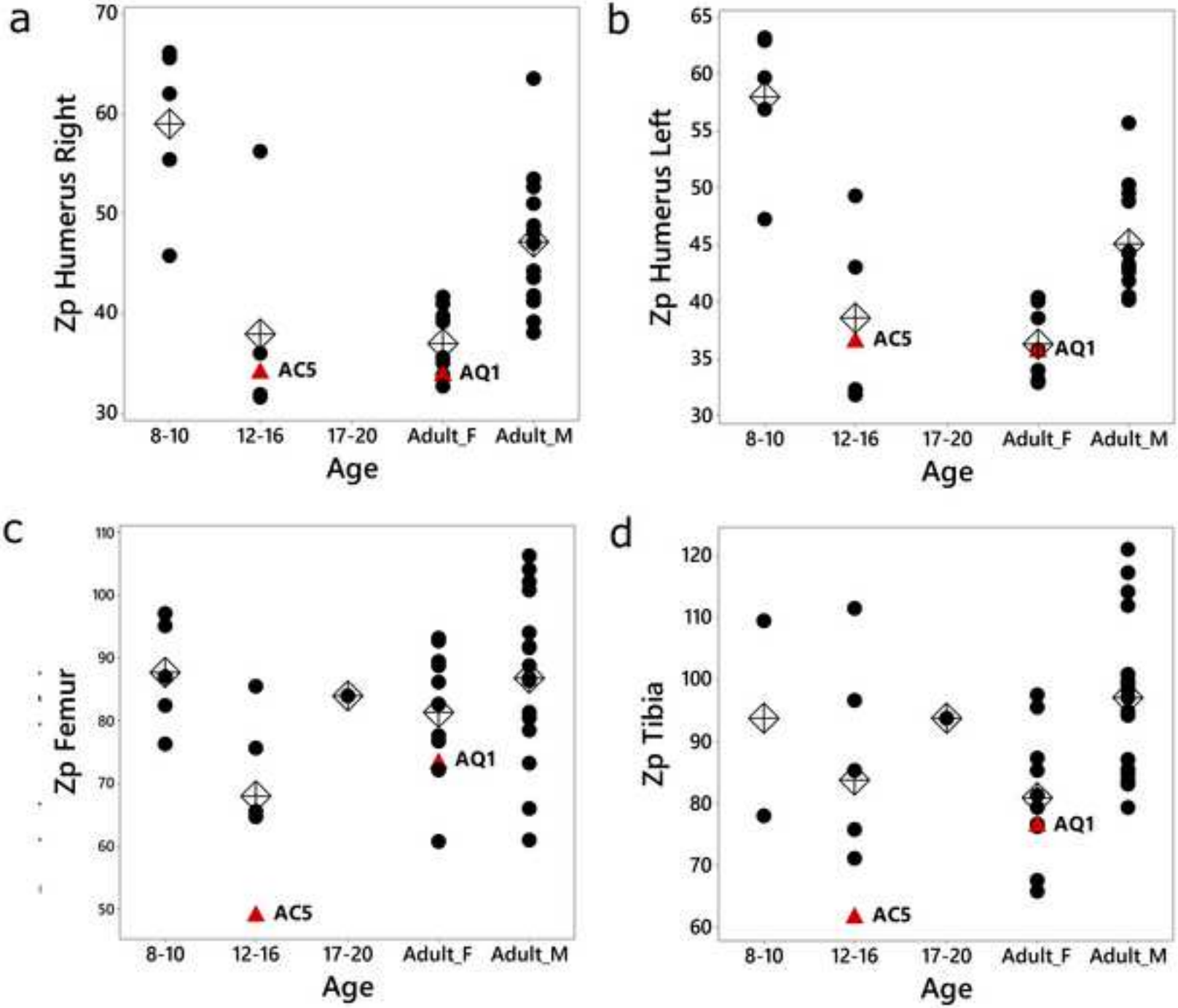
Figure

Click here to download high resolution image



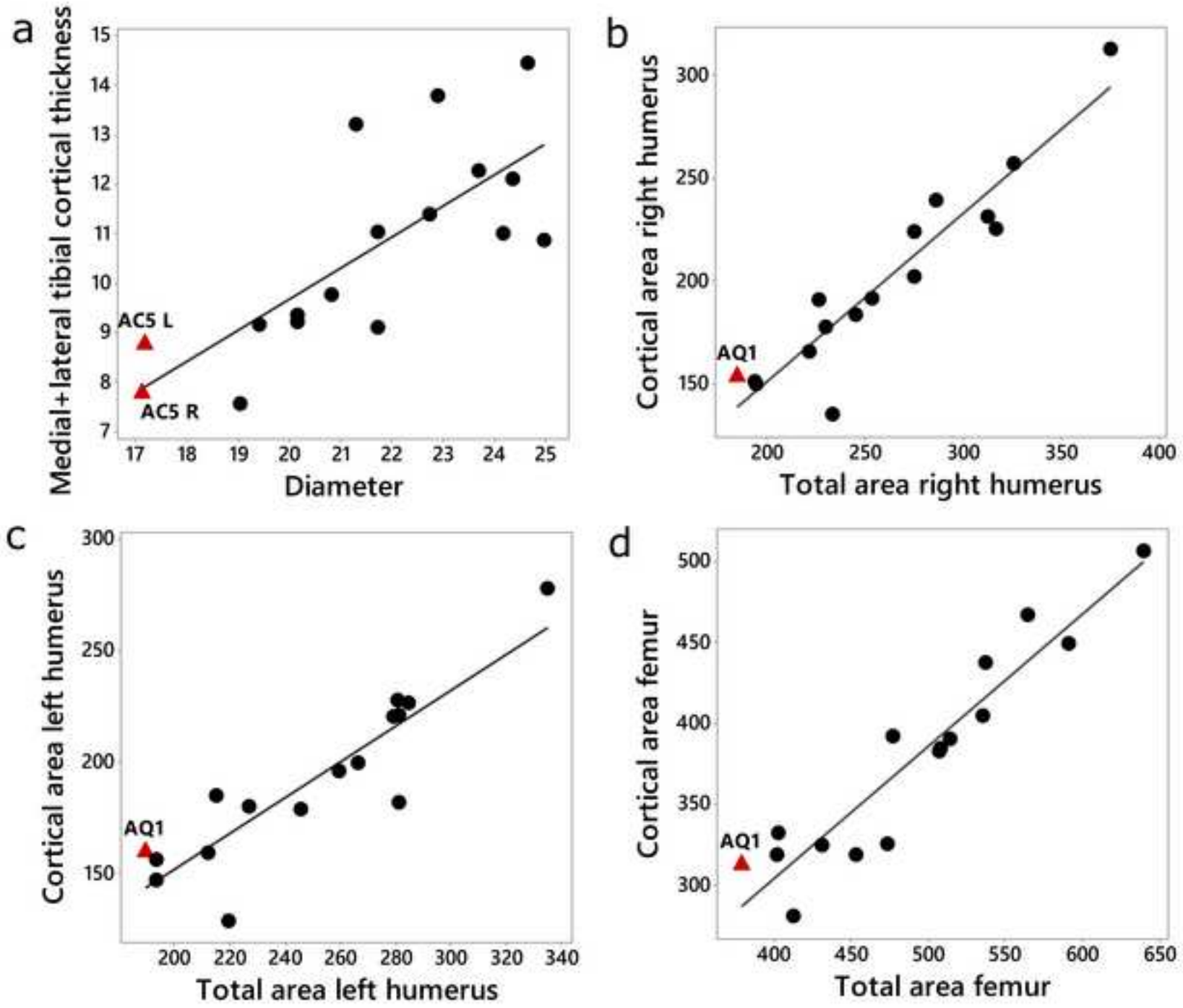
Figure

Click here to download high resolution image



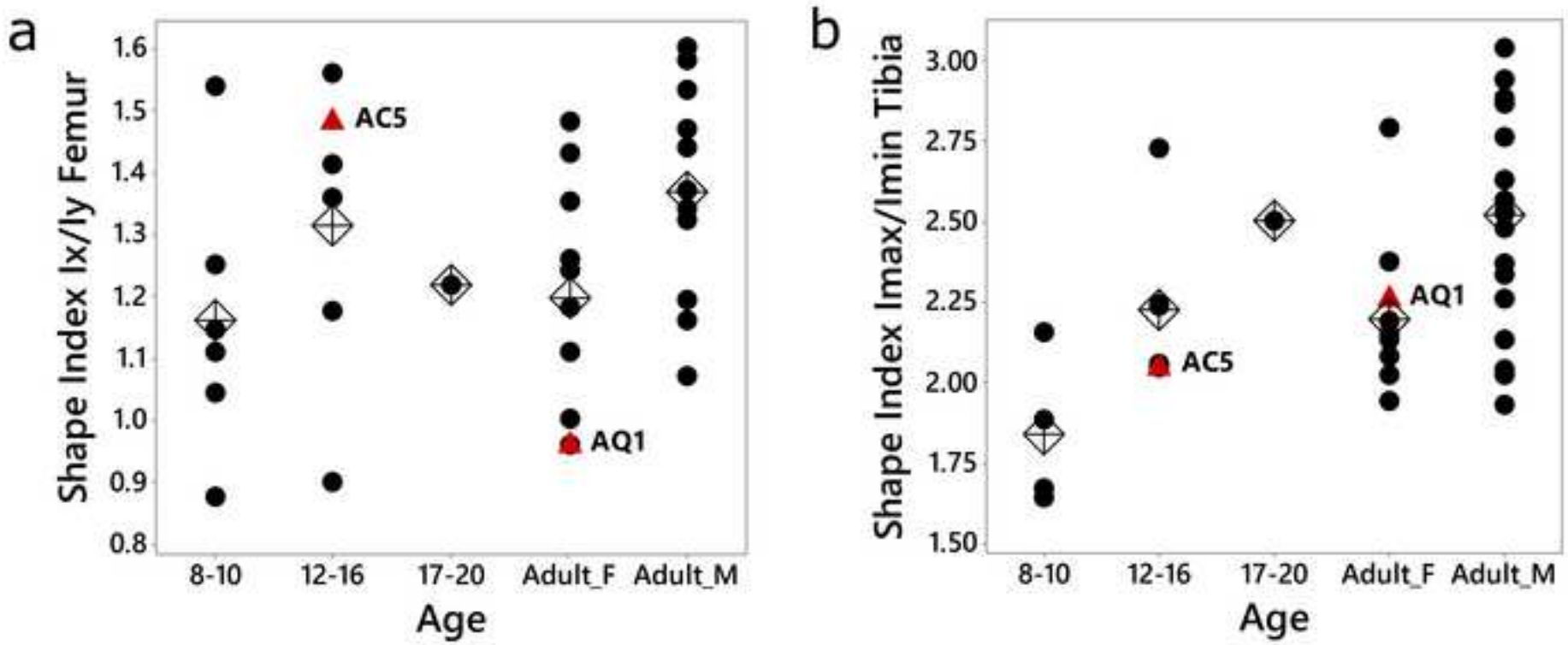
Figure

Click here to download high resolution image



Figure

Click here to download high resolution image



Tables

Site	Sex	Age	CSG Method	CSG Data Source	Bones Included	Museum
Arene Candide[1] 13	IND	8-10	Solid CSG	Present study	HR HL F T	Finale Ligure
Pollera 6623	IND	8-10	Solid CSG	Present study	HR HL F	Florence
Pollera 6682	IND	8-10	Solid CSG	Present study	HR HL F	Florence
Pollera 6687	IND	8-10	Solid CSG	Present study	HL F T	Florence
Pollera 2 (20 Pegli)	IND	8-10	SolidCSG	Present study	HR HL F T	Genova Pegli
Pollera XI (110 Pegli)	IND	8-10	SolidCSG	Present study	HR HL F T	Genova Pegli
Pollera 6684	IND	8-10	SolidCSG	Present study	HR HL	Florence
Pollera 5	IND	12-16	SolidCSG	Present study	HR HL F T	Genova Pegli
Arene Candide 6731	IND	12-16	SolidCSG	Present study	HR HL F T	Florence
Arene Candide 5[2]	M?	14-16	SolidCSG	Present study	HR HL F T	Finale Ligure
Arene Candide 6621	IND	14-16	SolidCSG	Present study	HR HL F T	Florence
Bergeggi 1 (1 Pegli)	M?	14-16	SolidCSG	Present study	HR HL F T	Genova Pegli
Pollera 4 (34 Pegli)	M?	14-16	SolidCSG	Present study	HR HL F T	Genova Pegli
Arene Candide 1	M	17-20	SolidCSG	Present study	FT	Finale Ligure
Adults	**Sex**	**Age**	**CSG Method**	**CSG Data Source**	**Bones included**	**Museum**
Arene Candide E IV	F	Adult	LCM	Previous studies	HR HL F T	Genova Pegli
Arene Candide VIII	F	Adult	Pearson	Present study	F T	Finale Ligure
Arene Candide XII	F	Adult	LCM	Previous studies	HR HL F T	Finale Ligure
Arma dell'Aquila 1 (1 Zambelli)[2]	F	Adult	LCM	Previous studies	HR HL F T	Finale Ligure
Arma dell'Aquila V (5 Richard)	F	Adult	LCM	Previous studies	HR HL F T	Finale Ligure
Bergeggi 2 (5 Pegli)	F	Adult	SolidCSG	Previous studies	F T	Genova Pegli
Boragni 1	F	Adult	SolidCSG	Previous studies	HR F T	Finale Ligure
Pollera 1 Tinè	F	Adult	LCM	Previous studies	HR HL F T	Finale Ligure
Pollera III (12 Pegli)	F	Adult	LCM	Previous studies	HR HL F T	Genova Pegli
Pollera VII (14 Pegli)	F	Adult	LCM	Previous studies	HR HL F T	Genova Pegli
Pollera VIII (33 Pegli)	F	Adult	LCM	Previous studies	HR HL F T	Genova Pegli
Arene Candide 2 Tinè	M	Adult	LCM	Previous studies	HL F T	Finale Ligure
Arene Candide II (6 Pegli)	M	Adult	SolidCSG	Present study	HR HL F	Genova Pegli
Arene Candide III (5700 Pigorini)	M	Adult	Pearson	Present study	F T	Rome Pigorini
Arene Candide IV (5690 Pigorini)	M	Adult	Pearson	Present study	F T	Rome Pigorini
Arene Candide IX	M	Adult	LCM	Previous studies	HR HL F T	Finale Ligure

Arene Candide V (5733 Pigorini)	M	Adult	Pearson	Present study	F T	Rome Pigorini
Arene Candide VI (8 Pegli)	M	Adult	LCM	Previous studies	HR HL F T	Genova Pegli
Arene Candide VII (7 Pegli)	M	Adult	LCM	Previous studies	HR HL F T	Genova Pegli
Arene Candide X (E VI Pegli)	M	Adult	LCM	Previous studies	HR HL F T	Genova Pegli
Arma dell'Aquila II (1 Richard)	M	Adult	LCM	Previous studies	HR HL F T	Finale Ligure
Arma dell'Aquila III (2 Richard)	M	Adult	Pearson	Present study	F T	Finale Ligure
Bergeggi II (3 Pegli)	M	Adult	SolidCSG	Previous studies	HR HL F T	Genova Pegli
Bergeggi III (4 Pegli)	M	Adult	SolidCSG	Previous studies	HR HL F	Genova Pegli
Boragni 2	M	Adult	SolidCSG	Previous studies	HR HL F T	Finale Ligure
Pian del Ciliegio 1	M	Adult	SolidCSG	Present study	HR HL F T	Finale Ligure
Pollera I (13 Pegli)	M	Adult	LCM	Previous studies	HR HL F T	Genova Pegli
Pollera II (10 Pegli)	M	Adult	LCM	Previous studies	HR HL F T	Genova Pegli
Pollera IV (6246 Pegli)	M	Adult	LCM	Previous studies	HR HL F T	Genova Pegli
Pollera VI (30 Pegli)	M	Adult	LCM	Previous studies	HR F T	Genova Pegli
Pollera X (32 pegli)	M	Adult	SolidCSG	Previous studies	HL T	Genova Pegli

Table 1 – Neolithic individuals from Liguria studied here: catalogue name (in parenthesis other denominations), sex, age, CSG method used (SolidCSG: Sparacello and Pearson, 2010; LCM: Latex Cast Method, O'Neill and Ruff, 2004; Pearson: Pearson, 2006; Pearson and Sparacello, In Press), CSG data source (previous studies: Marchi et al., 2006, Marchi 2008; Sparacello et al., 2011), skeletal elements included in the CSG study (HR, right humerus; HL, left humerus; F, femur; T, tibia), and location of the remains. Non-adults are ordered based on age at death; adults are in alphabetical order of the site, by sex. [1] Note that there is another skeletal series from Arene Candide belonging to the Upper Paleolithic, and numbers might overlap. In this table, we are referring exclusively to the Neolithic individuals. [2] Individuals showing skeletal signs of tuberculosis.

	PO 5	AC 6731	AC 6621	**AC 5[1]**	BER 1	PO 4	AC1	**AQ 1[1]**	Adolescents 12-16				Adults								
AGE	12-16	12-16	14-16	**14-16**	14-16	14-16	17-20	**Adult**					Males				Females				T-Test[4]
SEX	IND[2]	IND	IND	**M?**	M?	M?	M	**F**	N	-2SD	Mean	SD[3]	N	-2SD 10%ile	Mean	SD	N	-2SD 10%ile	Mean	SD	
Zp Humerus R	31.58	-	31.79	**33.96**	35.97	56.26	-	**33.75**	4	15.40	38.9	11.75	14	34.63 39.16	47.09	6.73	8	30.37 32.65	37.27	3.45	***
Zp Humerus L	32.32	-	37.79	**36.49**	43.00	49.33	-	**35.66**	4	22.01	39.11	8.55	15	35.99 40.24	45.07	4.54	7	29.92 32.89	36.37	3.23	***
HUMBA[5]	2	42.22	2.52	**9.72**	24.87	17.27	-	**4**	5	0	17.78	16.8	13	0 4.92	15.92	11.29	7	0 0.31	5.53	5.36	*
Zp Femur	65.58	-	75.58	**48.78 <**	64.67	85.56	83.98	**73.22**	4	53.23	72.85	9.81	17	60.83 65.89	86.73	12.95	10	61.30 66.32	82.00	10.35	NS
I_x/I_y Femur	1.56	0.9	1.17	**1.48**	1.41	1.36	1.21	**0.96**	5	0.59	1.24	0.33	12	1.03 1.16	1.37	0.17	9	0.86 0.96	1.22	0.18	*
Zp Tibia	75.63	111.58	85.39	**61.50**	71.16	96.66	93.73	**76.20**	5	55.33	88.09	16.38	17	71.51 83.03	97.20	12.84	10	60.16 66.61	81.23	10.53	**
I_{max}/I_{min} Tibia	2.23	2.05	2.72	**2.05**	2.04	2.24	2.50	**2.25**	5	1.71	2.26	0.28	19	1.81 2.02	2.52	0.36	10	1.70 1.98	2.19	0.24	*
Body Mass	41.65	42.65	44.61	**38.79**	42.78	44.44	57.32	**48.75**	5	40.70	43.22	1.26	19	50.61 53.37	62.76	6.07	10	41.23 45.29	53.25	6.01	***
Stature	142.29	-	150.64	**136.40**	141.61	136.96	154.84	**144.05**	4	131.49	142.88	5.69	19	147.97 153.06	159.68	5.86	10	140.11 142.36	150.55	5.22	***
BMI[6]	20.57	-	19.66	**20.85**	21.33	23.69	23.91	**23.49**	4	17.86	21.31	1.73	18	20.41 21.95	24.62	2.10	10	19.42 21.28	23.46	2.02	NS

Table 2 – Individual values of variables considered in this study for adolescent individuals compared to the sample statistics from the adult sample. Z_p is standardized by bone length × body mass. Indices are derived from non-standardized bending moments. The individuals showing skeletal signs of tuberculosis, i.e. the adolescent Arene Candide 5 and the adult Arma dell'Aquila 1, are in bold. The 'lower than' symbol indicates whether the value is below two standard deviations from the mean, or below the 10th percentile (this comparison has not been performed for non-adult body mass, stature, and BMI, because the differences are due to ontogeny). [1] Individuals showing skeletal signs of tuberculosis; [2] IND: sex not determinable; [3] SD: standard deviation; [4] Parametric T-Test comparing adult males and females; [5] HUMBA: humeral bilateral asymmetry [(max J – min J)/ min J] x 100; [6] BMI: body mass index [body mass (kg) / stature^2 (m^2)]; statistical significance: NS: non-significant, *: P<0.05, **: P<0.01, ***: P<0.001.