

Title: The effect of terrain on enthesal changes in the lower limbs

Authors: Maria Alejandra Acosta^{1,2,4}, Charlotte Yvette Henderson^{3,4}, Eugenia Cunha^{1,2,4}

Affiliations: ¹Laboratory of Forensic Anthropology, University of Coimbra, Calçada Martim de Freitas, 3000-456, Coimbra, Portugal.

²Centre for Functional Ecology, University of Coimbra, Coimbra, Portugal.

³CIAS - Research Centre for Anthropology and Health, University of Coimbra. Calçada de Martim Freitas, 3000-456, Coimbra, Portugal.

⁴Department of Life Sciences, University of Coimbra. Calçada Martim de Freitas, 3000-456, Coimbra, Portugal.

Email addresses for each author: Acosta MA. maacostav@gmail.com, Henderson CY c.y.henderson@uc.pt, Cunha E cunhae@uc.pt

Corresponding author: Acosta, MA

Corresponding author's address: Laboratory of Forensic Anthropology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456, Coimbra, Portugal. maacostav@gmail.com

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Abstract

One of the main factors involved in enthesal changes (EC) aetiology may be related to the physiological limits of biomechanical loading fixed during bone development, such that higher load during childhood and the adolescent growth spurt leads to a lower frequency of EC during adulthood. In this sense, it is possible that ECs may be related to overloading beyond an individual's normal physiological limits as established during childhood and adolescence.

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This meta-analysis tested this aetiological possibility by studying the influence of terrain on the entheses of the lower extremities. The hypothesis is that individuals who inhabited rugged terrain have lower EC than those living in flat terrain. This is because biomechanical loads associated with rugged terrain will lead to a higher normal capacity (defined during skeletal development) mitigating the probability of overloading compared to those living in flat terrain who will therefore have a higher frequency of ECs. To test this, papers reporting EC frequencies in the lower limbs were analysed alongside the local terrain. Terrain was defined into two categories: flat or rugged based on altimetry profile, i.e. the average elevation gains and losses along four specific paths (North-South, East-West, Northwest-Southeast, Southwest-Northeast). Odds ratios were calculated to compare rugged and flat terrain.

The overall results are consistent with the hypothesis that overloading is a factor in EC aetiology. However, when the analysis is conducted by sex and side, this general trend does not always occur. Limitations such as the lack of standardized age ranges could be affecting the outcome, i.e. older individuals have a higher frequency of ECs. The findings of this analysis suggest that the theoretical assumptions associated with the cause of ECs require further testing and evaluation.

Keywords: Locomotion, rugged, flat, meta-analysis, activity-related changes, functional adaptation

1.1 Introduction

Skeletal features associated with repetitive movements are still the most commonly used evidence to infer occupation in past populations. Division of labour based on sex, age, and social status are amongst the most frequent factors used to understand different cultural and socioeconomic dynamics (e.g. al-Oumaoui et al., 2004; Alves Cardoso, 2008; Henderson et al., 2013; Havelková et al., 2011, Hawkey and Merbs, 1995; Milella et al., 2012; Villotte et al., 2010). Environmental factors such as microclimate, natural resources, and terrain are often overlooked or dismissed but should be accounted for, as they can influence the performance of physical activities and the impact of biomechanical loads on the skeleton (al-Oumaoui et al., 2004; Dutour, 1986; Lukacs and Pal, 2003; Marchi et al., 2011). The analysis of ECs has been amongst the main skeletal indicators that support inferences of activity performance. However, research over the last two decades has shown that the relationship between ECs and physical activity is more complex than previously presented and more greatly affected by other factors such as age, sex, and body size (Jurmain et al., 2012; Milella et al., 2012; Niinimäki, 2011; Weiss et al., 2012). Furthermore, a complete understanding of the aetiology of the different types of EC and standardization of methodology are essential to achieve accurate inferences of the activity-patterns in past populations.

Current theoretical bases of EC analysis state that: i) ECs are all observable changes on the normal surface of the enthesis, i.e. where muscle, tendons, ligaments, and similar structures attach to the bone (Benjamin et al., 1986; Jurmain et al., 2012; Villotte and Knüsel, 2014; Villotte et al., 2016). ii) ECs are thought to be associated with repetitive movements (Hawkey and Merbs, 1995); iii) ECs are expressions of a multifactorial process that involves key factors such as ageing, hormones, body size, and biomechanical loads (Cunha and Umbelino, 1995; Jurmain et al., 2012; Milella et al., 2012; Niinimäki 2011; Weiss et al., 2012); iv) the amount of contribution of each of the above factors is unknown, although ageing appears to be a dominant factor (Alves-Cardoso and Henderson, 2013; Michopoulou et al., 2015, 2016; Milella et al., 2012; Niinimäki, 2011). In accordance with these premises, it is expected that ECs occur more frequently in individuals exposed to repetitive biomechanical loads than individuals with no such exposure or low physical activity levels. However, studies carried out on identified skeletal collections demonstrate that this assumption is not always correct (Cunha and Umbelino, 1995; Alves-Cardoso, 2008; Alves Cardoso and Henderson, 2010, 2013; Michopoulou et al., 2015, 2016 Milella et al., 2012; Henderson and Nikita, 2015) and we may be relying on erroneous assumptions or incomplete information.

Henderson (2013) conducted a meta-analysis suggesting that ECs could be related to the level of adaptation to loading of the musculoskeletal system, such that better adaptation to biomechanical load leads to a lower frequency of ECs. She tested the effect of subsistence strategy on ECs under the hypothesis that hunter-gatherers are better adapted to their lifestyle than agricultural and industrial populations. Henderson found the lowest EC scores in agricultural populations and the highest EC scores in industrial populations. While Henderson's original hypothesis was not fully supported by her results, she and other authors (Henderson, 2013; Henderson et al., 2013b; Jurmain et al., 2012) have noted methodological limitations and theoretical gaps that require further development. Specifically, the problems of considering the ageing effect.

The general configuration of land surface is a determining factor for environmental systems on which human groups have an impact and, in turn, are also impacted (Huggett 2010). Studies have shown that performing activities in rugged terrain influences the shape of diaphyses and increases the robusticity of the lower limbs (Marchi et al., 2006, 2011; Sparacello and Marchi, 2008, 2014). Although, diaphyseal shape and size of long bones and ECs are skeletal markers related to biomechanical load, the covariance between them is low (maximum R square of 0.3, Niinimäki, 2012), suggesting that physical activity is one of many factors involved in EC (Bridge, 1997; Michopoulou et al., 2015, 2016; Niinimäki, 2012; Weiss, 2003). Therefore, the relationship between terrain and ECs is not fully understood and has not been explored in depth (al-Oumaoui et al., 2004; Dutour, 1986; Lukacs and Pal, 2003; Weiss, 2014).

Following Henderson (2013), this meta-analysis uses terrain to explore whether ECs are related to overload as opposed to a result of normal load itself. The amount of normal load, that is, within the physiological limits or baseline of maximum load that an individual can support without affecting the bone, is determined by the characteristics and amount of activity performed during bone development and maturation (Bass et al., 2002; Pearson and Lieberman 2004). It is also possible that equivalent load levels must be regularly maintained after maturation to retain this capacity. Clearly, stating that overload may be an important factor associated with ECs neither implies that ECs are pathological conditions, nor that the absence of ECs is an indication of the absence of biomechanical loads. However, it implies that the morphology of the entheses surface are altered once the amount and characteristics of the biomechanical load performed during adulthood exceed the threshold set during skeletal development. We tested the hypothesis that individuals who inhabited rugged terrain have adapted their lower limbs to the biomechanical loads and show lower EC frequency in relation to those living in flat terrain.

2.1 Materials and Methods

Studies on musculoskeletal stress markers and enthesal changes were searched for in the Google Scholar server between the 23rd of February and the 13th of July of 2015. Only entheses of the lower limb were included as this is the limb affected by terrain during normal locomotion. Data sets from five papers and four PhD dissertations were collated (al-Oumaoui et al., 2004; Alves-Cardoso, 2008; Doying, 2010; Gresky et al., 2015; Henderson et al., 2013; Myszka and Piontek, 2012; Ponce, 2010; Rojas-Sepulveda, 2009; Speith, 2014).

While more than one hundred studies were found online, only nine studies presented frequencies of EC in the lower limb entheses (Table 1). Doying's paper (Doying, 2010) was included in the general trend analysis, but removed from the sex and side analysis because the results were neither separated by sex nor by side. Eighteen entheses from the lower limbs were collected. Eleven of the eighteen entheses were shared between reports of archaeological sites located in rugged terrain and flat terrain and were thus relied upon for this analysis.

Hawkey and Merbs (1995) was the most common recording method used in these reports, but the lack of methodological agreement has either promoted the creation of new methods (Myszka and Piontek, 2012), or has simplified the recording protocol to presence-absence of ECs, regardless of type or severity (al-Oumaoui et al., 2004; Gresky et al., 2015; Henderson et al., 2013; Ponce, 2010; Speith, 2014). Despite the heterogeneity of recording, all the authors, except for Myszka and Piontek 2012, reported or summarized the results in terms of EC presence-absence. Myszka and Piontek proposed a 3-degree rating system, in which 1 is the faintest score. For this report EC score "1" was standardized as "absence" and scores "2" or "3" as "present".

All lower limb entheses presented by authors were pooled by sex and side and then standardized using the name of the muscle involved.

Three more studies reporting the results in means were found (Schrader, 2015; Takigawa, 2014; White et al., 2000). However, the Schrader report had a different scale, and Takigawa pooled left and right data. Therefore, proper comparisons between the studies, and identification of patterns associated with type of terrain could not be done, and these reports were therefore excluded from this meta-analysis.

Classification of the terrain was carried out based on the altimetry profile of the terrain around the archaeological sites. The altimetry profile is the average of the sum of elevation gain and loss along four specific paths (North-South, East-West, Northwest-Southeast, Southwest-Northeast); quantifying the roughness of the relief associated with each skeletal set. The starting altitude does not impact on this value. Such profiles were assessed using a function of Google Earth version 6.2, and following the protocol developed by Sparacello and colleagues (2014). Their protocol classifies three types of terrain: flat (0m - 500m), moderately-hilly (500m - 1000m), and hilly-mountainous (1000-1500). However, the purpose of this analysis is to identify the effect of two types of terrain, thus a 500m altimetry cut-off point was set to differentiate flat from rugged terrain where all sites falling below this point were classified as flat and those above it as rugged. This cut-off point was set considering the classification proposed by Sparacello et al. 2014, and the altimetry profile of the populations under analysis. Data of skeletal remains from nineteen archaeological sites were analysed, within which four were classified as rugged terrain and fifteen as flat terrain.

Limiting the categories of terrain to only flat and rugged when attempting to identify the effect of different impacts on the human musculoskeletal system may fail to account for other important factors such as load carriage (Simpson et al., 2011), speed (den Otter et al., 2004; Lui et al., 2008; Neptune et al., 2008), slope (Franz and Kram, 2012), and distance. This is further complicated when characterizing archaeological landscape as physical shapes and the distribution of resources may have changed (Carson, 2011; Pott et al., 1999). Moreover, the relocation of human settlements for any reason e.g. trade, lack of natural resources, conflicts, or natural disasters could also have an impact on the musculoskeletal system, either because the physical demands during the last years of life were different from those which the individuals had been prepared for or had adapted to since adolescence, or because the sites where they were buried have different land surfaces to where they inhabited. Despite the limitations, this standardised categorization of flat/rugged was followed in this study because it aims to understand trends of ECs when interacting with these two different types of land surface configurations.

All the data collected were reported in frequencies. All missing data were removed. Odds ratio (OR) with their confidence intervals and p-values (95%) (calculated using standard formulae Szumilas, 2010) were used to calculate the probability that EC occurred less commonly in rugged compared to flat terrain sites (Bond et al., 2003; Field and Gillett, 2010; Nakagawa and Cuthill, 2007). Large confidence intervals indicate a low precision of the OR. P-values were calculated to assist the interpretation of the OR and its confidence intervals, highlighting where the confidence intervals do not span the null value.

Odds ratios were calculated for all right, all left, and pooled left and right data. Statistical analysis and graphs were calculated in Microsoft Excel 2011.

3.1 Results

A total of 896 individuals comprise the rugged terrain sample, and 1012 individuals comprise the flat terrain sample (Table 1). These are acceptable sample sizes, but it becomes an issue for some entheses in the flat sites, e.g. the triceps surae, where the sample sizes are low (see Table 2, and Table 3) and the trends may be affected.

Frequencies of EC are considerably diverse between entheses- for some entheses there are very low frequencies, e.g. gastrocnemius; whereas in others they seem to be more common in both terrains, e.g. the hamstrings (Fig. 1). The general trend, regardless of sex and anatomical side, is that flat terrain inhabitants present higher EC expression than rugged terrain inhabitants. Seven out of eleven variables (64%) support the hypothesis that individuals living in rugged terrains have higher physiological limits to respond to biomechanical loads than those living in flat terrains. However, deeper analysis enhances the difference in trends depending on the sex, and the way the sides were recorded, i.e. each side separated versus right and left pooled.

OR were calculated for all the entheses reported, except for quadriceps femoris in both left and right side for males and iliopsoas pooled left and right side for females. Those OR could not be calculated because sample sizes were small and not representative (either all individuals showed ECs, or none of them showed ECs). The general trend of OR (71%) indicated a higher frequency of EC in flat terrain than in rugged terrain. The odds of higher frequencies of ECs in individuals from flat terrain were statistically significant for the gastrocnemius, the gluteus maximus, the linea aspera, the patella tendon, and the hamstrings entheses in both female (Table 2) and male samples (Table 3).

Enthesal changes in the female sample were higher in the flat terrain individuals than in the rugged terrain individuals (68% of the variables), eleven out of seventeen of those variables showed statistically significant results (Table 2). In addition, ECs of the right gluteus medius (OR 3.60, 95% CI: 1.36-9.52) were statistically significant,

indicating that females from flat terrain have higher odds to show more frequency of ECs than the females living in rugged terrain. The opposite trend was evidenced in the left and right triceps surae. In this enthesis the flat terrain females had lower frequencies of EC than the females from rugged terrain. The right and left sides had different trends in the iliopsoas enthesis: the right side showed higher frequencies in the flat terrain, but the left side did not. Pooled right and left data shows opposite trends of the frequencies per side in which female individuals from rugged terrain have higher frequencies of ECs (71%) in most of the entheses analysed. However, none of the results of pooled right and left side were statistically significant.

The sample of males showed that most the entheses have higher odds of showing frequencies of ECs in the flat terrain than the rugged terrain individuals (73% of the variables); sixteen of those are statistically significant (Table 3). The results of the triceps surae enthesis (left OR 7.98, 95% CI, 1.69-37.68; right OR 4.21, 95% CI: 1.09-16.23) are statistically significant and showed the opposite trend than the female sample. The odds of rugged terrain individuals showing greater frequencies of ECs were evidenced for both the left and right iliopsoas (OR 0.67, 95% CI: 0.22-1.99; OR 0.65, 95% CI: 0.22-1.93). Within the pooled right and left data, 63% of the entheses showed higher frequencies of ECs for rugged terrain than for flat terrain individuals, two of those were statistically significant.

4.1 Discussion

The objective of this meta-analysis was to test the effect of terrain on ECs in the lower limbs. Results of left and right side in both female and male individuals are consistent with the hypothesis that individuals living in rugged terrain have less overloading (lower frequencies of ECs), probably due to high levels of loading during skeletal development. Yet individuals from rugged terrains showed trends of higher frequency in some entheses. This could be due to other non-terrain factors such as subsistence strategy (Table 1), age, sexual dimorphism, the relocation of settlements, or the biomechanical overload itself.

The muscles required for locomotion in both terrains are mostly the same, but the intensity of the biomechanical loads differs (Lay et al., 2007; Massaad et al., 2007). Studies have found that the activity of the gluteus maximus, the rectus femoris, the vastus medialis, and the gastrocnemius increases significantly when walking on a slope (Franz and Kram, 2012; Lay et al., 2007). Our results of ECs in the gluteus maximus, the gastrocnemius, and the hamstrings (as an antagonist to the quadriceps) for both female and male individuals have a higher frequency in flat terrain and the results reached statistical significance, therefore those entheses support the hypothesis of this meta-analysis.

The linea aspera is the attachment site of multiple muscles mainly responsible for hip movement and the patella tendon functions to maintain the patella in the right position and assist in the bending of the leg. These two entheses could be indirectly associated with activities that involved hip and knee movements, e.g. locomotion. In this study the results from both sides and for both sexes support the hypothesis that rugged terrain individuals have set wider physiological limits than individuals living in flat terrain.

The pooled left and right data of male individuals showed the opposite trend for the quadriceps femoris and the triceps surae. The results of these two entheses do not support the hypothesis. However, contradictory findings are observed on the left and the right triceps surae recorded independently, where EC have higher frequency in male individuals from flat terrain, thereby supporting the hypothesis. This contradiction may be associated with methodological limitations, such as small sample sizes. It also highlights the need to standardize recording and reporting methods. Further comparisons are required, to increase the sample size.

Males from rugged terrain also showed the tendency to have higher EC in the iliopsoas entheses than males from flat terrain. Iliacus and psoas are the muscles responsible for rotation and flexion of the hip, and the overloading of these muscles is related to changes in the centre of balance when walking speed increases (Lui et al., 2008; Neptune et al., 2008). Rugged terrain may stimulate the reduction of the average locomotion speed; thus, the overload of this muscle has higher odds to be observed in the inhabitants of rugged terrain than in flat terrain.

Two important differences are observed between the male and female samples. The left and right side of the triceps surae females from rugged terrains have higher frequencies of EC than those from flat terrains; whereas the males follow the normal trend, although sample sizes in both sexes are quite small which could affect the trends. The triceps surae muscle is involved in plantar flexion, stabilizes the ankle during locomotion and during power activities such as jumping. Thus, EC (if biomechanically modulated) are most likely due to the performance of activities requiring more stability of the foot than their physiological limits were prepared for. On the other hand, both males and females showed higher frequencies of EC within individuals from flat terrain for the gluteus medius entheses. However, the odds of EC were statistically significant in the opposite sides, the right side in females and the left side in males. The difference in sex patterns may reflect either the expression of ECs caused by sexual dimorphism, division of labour from a young age, or intensification of activities during adulthood, and such activities are likely associated with the resources and needs of each terrain.

Age is one factor that has proven to be associated with ECs and has become an obligatory element to consider in this sort of analysis (Henderson and Alves Cardoso, 2013; Niinimäki, 2011; Milella et al., 2012). Regrettably, due to the limited information provided in the literature used in this meta-analysis, it was not feasible to control age ranges, which could result in erroneous or biased results and interpretations (discussed in Henderson, 2013). Nevertheless, analysis of bilateral asymmetries may help to reduce the effect of age on ECs and allow for the identification of activity-patterns, i.e. the age affects the entheses bilaterally, but the biomechanical load mainly impacts the dominant side. The asymmetries found in this meta-analysis showed that the right side had higher frequencies of scores than the left within each terrain. Left side dominance in the lower limb has been described in the literature (see review in Sadeghi et al., 2000). The higher frequencies on the right side may further support the hypothesis in this study.

In interpreting these results, it is important to consider the limitations that restricted the comparison between data sets. The most relevant limitations were the lack of standardization of recording methods, which has been previously pointed out by other authors (Henderson et al., 2013b; Villotte et al., 2010; Villotte et al., 2016); the variation of the entheses recorded by each author and their anatomical limits, i.e. some are combined into one score for multiple entheses due to their proximity, e.g. semitendinosus and semimembranosus; results are reported using different statistics or scales and age was not controlled for.

The need to standardize the recording method of ECs has been widely discussed, particularly because of the anatomical differences between fibrous and fibrocartilaginous entheses (see discussion in Henderson et al., 2013b; Villotte et al., 2010; Villotte et al., 2016). Although, significant advances have been achieved (Henderson et al., 2013b, 2016; Mariotti et al., 2004, 2007; Villotte, 2006, 2009) ECs are still not recorded or reported with a unified method making proper comparison and meta-analysis difficult to accomplish (Henderson, 2013). Also, the names of entheses should be standardized according to the muscle involved instead of the anatomical part of the bone, because some locations, such as the greater trochanter and the ischial tuberosity, have more than one muscle, ligament, or tendon attached to them, which may result in confusion of which entheses have been recorded. Another approach would be to test agonist/antagonist muscles pairs (Villotte et al., 2014), but this is impossible due to the nature of the data reported and draws attention to the need to establish a basic set of entheses to be recorded in all studies (Henderson, 2015). Due to this lack of methodological conformity, the authors propose separation of frequencies by age, sex, and side when recording and reporting ECs given that both age and sex play a fundamental role in the expression of EC (Milella et al., 2012; Niinimäki, 2011; Weiss, 2007; Wilczak, 1998) and side differences are the only way to identify bilateral asymmetries. Using asymmetry within individuals rather than right and left side scores, may also remove the effect of age as both sides should be affected by age at the same rate. In fact, within

individual ratios the bilateral asymmetries have been shown not to be age related, e.g. the ratio of EC in the common extensor and the common flexor (Villotte and Knüsel, 2014), so patterns associated with physical activity may be identified.

Sample sizes in archaeological contexts are always an important limitation to be addressed. Small sample sizes with fewer recordable skeletons are the most common scenarios, and the lack of standardization of recording and reporting methods is enhancing this limitation. In this meta-analysis, the sample sizes of both terrains were quite large but the diversity of the entheses recorded and the reporting formats used by each author, e.g. pooled left and right data, pooled male and female individuals, significantly reduced the final sample sizes.

Research of the activity patterns of past populations should be the result of the analysis and interpretation of skeletal evidence, landscape characteristics, artefacts and other cultural material (Goodman and Leatherman, 1998). However, skeletal traits have taken the primary role in this type of analysis which may be contributing to incomplete or erroneous interpretations. It is essential to analyse activity patterns from all sources independently, and then contrast inferences based on skeletal evidence with the other factors. The understanding of the aetiology of different types of ECs is complicated, but can be achieved by looking carefully at all possible factors involved from skeletal development to the characteristics of the load (or overload) itself.

5.1 Conclusions

Enthesal changes are often considered indicators of repetitive loading, but evidence is emerging that overloading may be a causative factor i.e. it is likely that ECs are related to overload as opposed to a result of normal load itself, whereas EC could be more frequent in individuals that had exceeded the physiological limits set during skeletal development.

This study tested the hypothesis of whether individuals who inhabited rugged terrain have higher physiological limits to respond to biomechanical loads thus have lower odds of ECs in relation to those living in flat terrain. Using previously published literature, this study showed that general trends, in both males and females, supported the hypothesis. It is, however, important to compare left and right sides separately, even in the lower limb as there are differences in frequencies between sides. Sexes should also be considered separately.

The results of this analysis suggest that EC studies are extensive but require standardization of methods and reporting. Moreover, the theoretical assumptions associated with the aetiology of EC need further review and evaluation.

Additional studies that have standardized data and that control for age are required in order to understand the aetiology of the different types of enthesal changes. This will provide a stronger theoretical basis upon which results may be interpreted.

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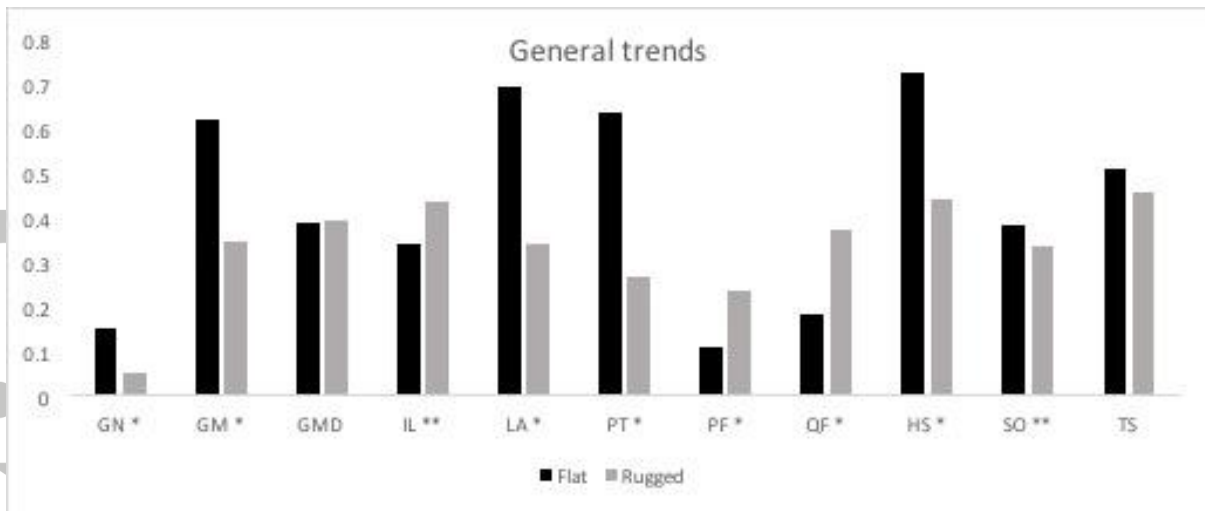


Table 1. Literature used in the meta-analysis classified by terrain.

Citation	Site	Sample size	Subsistence strategy	Terrain	Altimetry profile
Al-Oumaoui et al. 2004	La Carada	75	Agricultural	Flat	149.15
	Argar	83	Mixed	Rugged	790
	La torecilla	98	Agricultural	Flat	452.75
	Villanueva	57	Agricultural	Flat	470.15
	San Baudelio	29	Mainly herding	Flat	328.63
Alves-Cardoso 2008	Portuguese skeletal collections	603	Industrial	Rugged	526.75
	Maxwell Museum's Documented Skeletal Collection			Flat	
Doying 2010		69	Industrial	Flat	112.38
Gresky et al. 2015	Liushui	110	Hunter-gatherer	Rugged	1189.75
Henderson et al 2013	Church of St. Michael and St. Lawrence	18	Industrial	Flat	171.48
Myszka and Piontek 2012	Cedynia	201	Agricultural	Flat	85.38
Ponce 2010	Costal fishers sites	75	Fishing	Flat	115.47
	Inland agricultural sites	100	Agricultural	Rugged	511.73
Rojas-Sepulveda 2009	Ancón	116	Fishing/ trade	Flat	457.67
	Marin	41	Agricultural	Flat	366.22
	Panamá	18	Agricultural	Flat	130.45
	Sitio Sierra	19	Agricultural	Flat	40.22
	Soacha	91	Agricultural	Flat	283.13
Speith 2014	Tunja	99	Agricultural	Flat	315.85
	Niederstotzingen	9	Agricultural	Flat	180.6

Table 2. Results of the female sample

			Site (n)	Sample size (n)	EC (n)	%	Odds ratio	p value (OR)	Lower 95% CI	Upper 95% CI
Gastrocnemius	Left	Flat	6	105	17	16%				
		Rugged	1	298	15	5%	3.64	0.001*	1.75	7.60
	Right	Flat	6	113	16	14%				
		Rugged	1	300	23	8%	1.99	0.047	1.01	3.92
Gluteus maximus	Left	Flat	7	203	120	59%				
		Rugged	1	294	111	38%	2.38	0.000*	1.65	3.44
	Right	Flat	7	209	118	56%				
		Rugged	1	297	108	36%	2.27	0.000*	1.58	3.26
Gluteus medius	Left	Flat	1	18	12	67%				
		Rugged	2	338	163	48%	2.15	0.136	0.79	5.85
	Left & right	Flat	4	64	12	19%				
		Rugged	1	24	1	4%	5.31	0.119	0.65	43.27
	Right	Flat	1	21	15	71%				
		Rugged	2	327	134	41%	3.60	0.01	1.36	9.52
Hamstrings	Left	Flat	2	20	19	95%				
		Rugged	2	337	169	50%	18.89	0.004	2.50	142.70
	Right	Flat	2	23	18	78%				
		Rugged	2	338	166	49%	3.73	0.011	1.35	10.28
Iliopsoas	Left	Flat	2	26	8	31%				
		Rugged	1	48	18	38%	0.74	0.575	0.27	2.05
	Left & right	Flat	4	77	9	12%				
		Rugged	1	23	0	0%	NA	NA	NA	NA
	Right	Flat	2	30	14	47%				
		Rugged	1	49	22	45%	1.07	0.887	0.43	2.67
Linea aspera	Left	Flat	2	100	84	84%				
		Rugged	2	357	130	36%	9.17	0.000*	5.15	16.32

Patella tendon	Left & right	Flat	4	87	20	23%				
		Rugged	1	29	2	7%	4.03	0.072	0.88	18.44
	Right	Flat	2	108	91	84%				
		Rugged	2	360	114	32%	11.55	0.000*	6.58	20.29
	Left	Flat	2	81	63	78%				
		Rugged	2	339	63	19%	15.33	0.000*	8.49	27.69
Plantar fascia	Left & right	Flat	4	68	5	7%				
		Rugged	2	34	6	18%	0.37	0.125	0.10	1.32
	Right	Flat	2	94	78	83%				
		Rugged	2	339	58	17%	23.62	0.000*	12.86	43.37
	Left & right	Flat	4	56	2	4%				
		Rugged	2	51	6	12%	0.28	0.128	0.05	1.44
Quadriceps femoris	Left & right	Flat	4	40	6	15%				
		Rugged	1	18	3	17%	0.88	0.881	0.19	4.01
Soleus	Left	Flat	8	238	92	39%				
		Rugged	2	357	131	37%	1.09	0.641	0.78	1.52
Triceps surae	Left & right	Flat	4	83	11	13%				
		Rugged	2	46	9	20%	0.63	0.351	0.24	1.65
	Right	Flat	8	253	99	39%				
		Rugged	2	355	130	37%	1.11	0.540	0.80	1.55
	Left	Flat	1	2	1	50%				
		Rugged	2	276	145	53%	0.90	0.948	0.06	14.59
Left & right	Flat	4	59	7	12%					
		1	32	7	22%	0.48	0.214	0.15	1.52	
	Right	Flat	2	4	2	50%				
		Rugged	2	289	154	53%	0.88	0.904	0.12	6.31

Table 3. Results of the male sample

			Site (n)	Sample size (n)	EC (n)	%	Odds ratio	p-value (OR)	Lower 95% CI	Upper 95% CI
Gastrocnemius	Left	Flat	6	118	17	14%				
		Rugged	1	297	12	4%	4.00	0.000*	1.85	8.66
	Right	Flat	6	115	18	16%				
		Rugged	1	299	13	4%	4.08	0.000*	1.93	8.64
Gluteus maximus	Left	Flat	8	247	160	65%				
		Rugged	1	292	96	33%	3.75	0.000*	2.63	5.37
	Right	Flat	8	244	151	62%				
		Rugged	1	295	91	31%	3.64	0.000*	2.55	5.20
Gluteus medius	Left	Flat	3	21	16	76%				
		Rugged	2	313	104	33%	6.43	0.000*	2.29	18.04
	Left & right	Flat	4	75	19	25%				
		Rugged	1	29	9	31%	0.75	0.569	0.29	1.94
Hamstrings	Right	Flat	3	24	12	50%				
		Rugged	2	307	114	37%	1.69	0.217	0.74	3.89
	Left	Flat	3	26	19	73%				
		Rugged	2	313	116	37%	4.61	0.001*	1.88	11.30
Iliopsoas	Right	Flat	3	26	16	62%				
		Rugged	2	320	127	40%	2.43	0.034	1.07	5.53
	Left	Flat	3	27	17	63%				
		Rugged	1	32	23	72%	0.67	0.476	0.22	1.99
Linea aspera	Left & right	Flat	4	76	27	36%				
		Rugged	1	30	10	33%	1.10	0.842	0.45	2.69
	Right	Flat	3	24	13	54%				
		Rugged	1	31	20	65%	0.65	0.447	0.22	1.93
Patella tendon	Left	Flat	3	115	99	86%				
		Rugged	2	331	123	37%	10.46	0.000*	5.90	18.56
	Left & right	Flat	4	100	43	43%				
		Rugged	1	39	10	26%	2.19	0.061	0.96	4.97
Right	Flat	3	114	96	84%					
	Rugged	2	335	113	34%	10.48	0.000*	6.03	18.20	
Patella tendon	Left	Flat	2	85	71	84%	8.58	0.000*	4.63	15.91

	Left & right	Rugged	2	315	117	37%				
		Flat	4	85	20	24%				
	Right	Rugged	2	55	16	29%	0.75	0.472	0.35	1.62
		Flat	2	119	95	80%				
Plantar fascia	Left & right	Rugged	2	317	117	37%	6.77	0.000*	4.09	11.18
		Flat	4	81	13	16%				
		Rugged	2	68	13	19%	0.81	0.636	0.35	1.89
		Flat	4	63	13	21%				
Quadriceps femoris	Left & right	Rugged	1	28	15	54%	0.23	0.002	0.09	0.59
		Flat	9	268	102	38%				
Soleus	Left	Rugged	2	328	95	29%	1.51	0.019	1.07	2.21
		Flat	4	107	43	40%				
	Left & right	Rugged	2	72	17	24%	2.17	0.022	1.12	4.24
		Flat	9	261	114	44%				
Triceps surae	Right	Rugged	2	330	114	35%	1.47	0.024	1.05	2.05
		Flat	3	11	9	82%				
	Left	Rugged	2	269	97	36%	7.98	0.009	1.69	37.68
		Flat	4	80	41	51%				
Left & right	Left & right	Rugged	1	30	23	77%	0.32	0.019	0.123	0.83
		Flat	3	11	8	73%				
	Right	Rugged	2	276	107	39%	4.21	0.036	1.09	16.23
		Flat	4	80	41	51%				