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Mineralized area of the human rib cross-sections from early puberty until adulthood

J. M. López-Rey[1,2] | D. M. Doe[2] | O. Cambra-Moo[2] | A. González Martín[2] | D. García-Martínez[2,3,4,5]

[1]Paleoanthropology Group, Department of Paleobiology, Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid, Spain

[2]Laboratorio de Poblaciones del Pasado (LAPP), Department of Biology, Faculty of Sciences, Universidad Autónoma de Madrid (UAM), Madrid, Spain

[3]Physical Anthropology Unit, Faculty of Biological Sciences, Universidad Complutense de Madrid (UCM), Madrid, Spain

[4]Center for Functional Ecology—Science for People and the Planet (CFE). Laboratory of Forensic Anthropology, Centre for Functional Ecology, Department of Life Sciences, University of Coimbra (UC), Coimbra, Portugal

[5]Centro Nacional de Investigación sobre la Evolución Humana (CENIEH), Burgos, Spain

Correspondence

J. M. López-Rey, Paleoanthropology Group, Department of Paleobiology, Museo Nacional de Ciencias Naturales (MNCN-CSIC), Calle José Gutiérrez Abascal, 2, 28006 Madrid, Spain; Laboratorio de Poblaciones del Pasado (LAPP), Department of Biology, Faculty of Sciences, Universidad Autónoma de Madrid (UAM), Calle Darwin, 2, 28049 Madrid, Spain.

Email: jolopezr@mncn.csic.es

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Abstract

Ribs undergo numerous changes during growth and development. Although they occur both externally and internally, the latter are not as extensively documented during the transition from puberty to adulthood. Therefore, it is unknown how rib cross-sectional mineralized area changes during this period. To shed light on this issue, we micro-CT scanned ribs from each costal level belonging to 21 individuals equally distributed into three developmental groups: pre-pubescents, post-pubescents, and adults. Then we selected the cross section at the midshaft of each rib and measured its percentage of mineralized area. Our results show that adults have lower mineralized area in their rib cross sections than both pre- and post-pubescents, which is consistent with previous research. Between pre- and post-pubescents, mineralized area is greater in the latter from costal levels 1–8. We propose that this might respond to a peak of mineralized area happening during late puberty. Regarding the tendency of the data, the three groups show a U-shaped trend with two maximum values at costal levels 1 and 12 and a minimum value at levels 4–5. We suggest that greater values are located at the beginning and the end of the costal series due to the mechanical stress produced in these areas by the scalene muscles (ribs 1–2) and diaphragm (ribs 7–12) during breathing. Interestingly, the U-shaped trend is less pronounced in pubescents, whose central costal levels have relatively more mineralized area than that of adults due to ongoing maturation from the external to central costal levels.

KEYWORDS

adulthood, breathing, mineralized area, puberty, ribs

1 | INTRODUCTION

Ontogeny plays a determinant role in driving the morphology and functionality of human anatomy. Through growth and development, bones undergo changes in size and shape, as well as processes such as epiphyseal fusion and cranial suture closure. Since these processes can be used to infer the biological age of an individual, the study of ontogeny is crucial in disciplines like developmental anthropology. While the evolution of external skeletal morphology during growth and development has been extensively studied and described (e.g., Cunningham et al., 2016), internal bone changes that occur during ontogeny remain less explored, despite their comparable significance in understanding skeletal development.

In the past, destructive techniques were needed to access bone cross sections, limiting samples available for this type of research. These methods also required the processing of obtained tissues, which is highly demanding and time-consuming (Cambra-Moo et al., 2012, 2014; Goldman et al., 2009; Pitfield et al., 2017). However, these limitations can be overcome by introducing new workflows that include (micro-) CT scanning and digital tools into anthropological studies. Both improvements have enabled larger samples to be gathered without damaging the existing skeletal material and have facilitated faster analyses compared to those performed directly on tissues (García-Martínez et al., 2023; Goliath et al., 2022; Swan et al., 2020).

Regardless of the methodology, research on bone cross sections has concluded that although bone tissues undergo modeling and remodeling throughout life, the mineralized area in cross sections decreases from childhood to adulthood. These ontogenetic studies have been conducted mainly on long bones (Cambra-Moo et al., 2012, 2014; Goldman et al., 2009; Goliath et al., 2022; Pitfield et al., 2017; Swan et al., 2020). Regarding ribs, research has been scarce as only a handful of articles have studied the effects of ontogeny through the digital analysis of rib cross sections (Beresheim et al., 2019; García-Martínez et al., 2017, 2023; Holcombe et al., 2024). None of these papers, however, have tested how mineralization changes until adulthood on the full costal series.

For adults, López-Rey et al. (2022) showed that there is a U-shaped trend for cross-sectional mineralized area along the 12 rib levels. Greater mineralized area was found in levels 1–2 and levels 7–12, possibly due to the mechanical stress produced by the attachments of the scalene muscles and the diaphragm, respectively. Given that rib cross-sectional mineralized area is greater in the earlier stages of growth and development (Beresheim et al., 2019; García-Martínez et al., 2017), it is unknown whether the U-shaped trend described for adults would also be present in immature individuals. This question is especially interesting when considering the transition from child to adult during puberty, given that previous research found a greater rate of tissue reorganization during the pubertal growth spurt (Gilsanz et al., 1988; Kröger et al., 1993; Pearson & Lieberman, 2004). Therefore, the aim of this study is to gather a sample of individuals in different ontogenetic stages, from pre-pubescents to adults, to test the following hypotheses:

1. The area of mineralized tissues in the rib cross sections at the midshaft decreases gradually from early puberty until adulthood.
2. There is a U-shaped trend in the cross-sectional mineralized area of the costal series that is independent of the ontogenetical phase of the individual.

2 | MATERIALS AND METHODS

To carry out this study, we first established three groups based on skeletal development: pre-pubescents (between 8 and 11 years old), post-pubescents (between 17 and 25 years old), and adults (>30 years old). To gather a consistent sample, we selected the best-preserved costal remains of various individuals from several medieval Christian cemeteries from the northwestern area of the Iberian Peninsula. Costal remains belonging to these individuals lacked pathological or taphonomical alterations that could alter rib anatomy. This study included 7 pre-pubescent and 7 post-pubescent individuals from the cemetery of *Veranes* (Gijón, Asturias, Spain; Rascón Pérez et al., 2013), and 7 adults from the cemetery of *Marialba de la Ribera* (Villaturiel, León, Spain; Candelas González et al., 2016). While puberty status was determined using standard methods (Lewis et al., 2016; Shapland & Lewis, 2013, 2014), age-at-death was estimated using the permanent mandibular dentition (Liversidge & Marsden, 2010; Moorrees et al., 1963) and skeletal maturation (Lewis et al., 2016) when dental development was complete. For immature individuals, biological sex was assigned based on features of the pelvis (Bruzek, 2002; Phenice, 1969) and humerus (Bass, 2005). When all three bones of the pelvis were fused, the DSP method was employed (Brůžek et al., 2017; Murail et al., 2005). Table 1 provides more information about the chosen sample, which belongs to the osteoarcheological collections housed at the *Laboratorio de Poblaciones del Pasado* (LAPP, Department of Biology, Faculty of Sciences, *Universidad Autónoma de Madrid*, Spain).

For each individual, the best-preserved rib of each costal level was scanned via micro-CT at the *Centro Nacional de Investigacion sobre la Evolución Humana*

TABLE 1 Expanded information about the sample.

ID	Collection (UAM)	Sex	Age	Stage
47.1	Veranes (Asturias, Spain)	Male	10	Pre-pubescent
164		Female	10	
310		Male	9	
328		Female	11	
451		Male	10	
481		Female	10	
539		Female	8	
137	Veranes (Asturias, Spain)	Male	20–25	Post-pubescent
249		Female	17	
295		Male	20–25	
320		Male	20–25	
489		Female	20–21	
586		Female	18	
601		Female	19	
70.1	Marialba de la Ribera (León, Spain)	Male	≥ 30	Adult
87		Female		
102		Female		
120.1		Male		
149		Female		
178		Male		
221		Female		

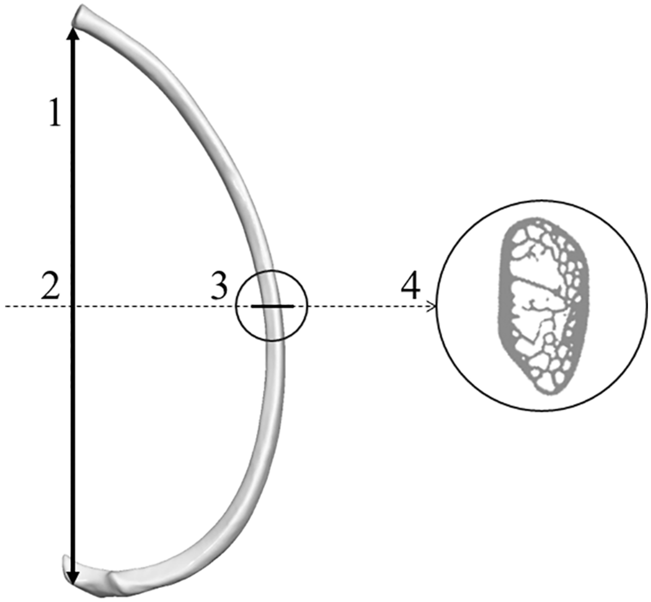

FIGURE 1 Extraction of the rib cross section at the midshaft from a seventh rib. We drew a tuberculo-ventral chord (1), calculated its bisection (2), selected the midshaft of the rib corpus (3), and extracted the corresponding cross section (4).

TABLE 2 Mean and standard deviation of the percentage of mineralized area of the rib cross sections per group and costal level.

Costal level	Pre-pubescent	Post-pubescent	Adult
1	82.78 ± 7.6	85.92 ± 6	76.84 ± 7.2
2	73.28 ± 4.9	75.14 ± 11.4	64.89 ± 9.3
3	71.42 ± 7.04	72.25 ± 10.2	61.78 ± 12.6
4	67.63 ± 4.7	70.54 ± 11.6	59.29 ± 7.7
5	68.29 ± 7.05	70.14 ± 10.5	59.01 ± 7.2
6	69.16 ± 7.7	72.93 ± 7.2	62.70 ± 7.2
7	71.81 ± 5.5	76.28 ± 7.1	61.88 ± 9.03
8	70.67 ± 5.8	74.69 ± 4.4	61.84 ± 11.8
9	75.85 ± 7.5	75.11 ± 4.5	65.96 ± 9.7
10	80.55 ± 6.04	79.61 ± 5.4	69.32 ± 7.5
11	83.24 ± 7.02	81.61 ± 5.7	72.22 ± 4.2
12	85.79 ± 9.3	86.60 ± 4.8	76.18 ± 6.4

(CENIEH) facilities, using a V|Tome|X s 240 equipment (GE Sensing & Inspections Technologies) with the following specifications: resolution = 90 μm; kV = 110; μA = 100. The final rib volumes were reconstructed and saved in DICOM format to extract their cross sections at the midshaft using Slicer v. 5.6.2 (Fedorov et al., 2012) as previously done by López-Rey et al. (2022). To sum up, we first drew a tuberculo-ventral chord, which is a straight line between the ventral margin of the costal tubercle to the ventral-most point of the sternal end of the rib (Franciscus & Churchill, 2002). Then we calculated its bisection and extracted the cross-section at the point where that bisection crosses the rib corpus, which we considered as the midshaft (Figure 1). Images containing these cross sections were saved in TIFF format and their percentages of mineralized area (% Min. Ar.) were subsequently calculated (Table 2, SOM Table S1) using Fiji-Image J v. 2.1.0/1.53s (Schindelin et al., 2012). For that purpose, this software provides a threshold that allows differentiation between bone tissue (mineralized area, including both cortical and trabecular) and soft tissue (non-mineralized area). Knowing the total area of each cross section, we determined the % Min Ar. of the sample and then studied its graphical distribution using line plots with error bars (Figure 2). We also performed permutation tests (10,000 permutations) on the % Min. Ar. to check whether there were significant differences between the three groups along the costal series (Table 3). Eventually, we chose an example individual from each group and depicted their rib cross sections to highlight key differences among age cohorts per costal level (Figure 3). Statistical analyses were performed in

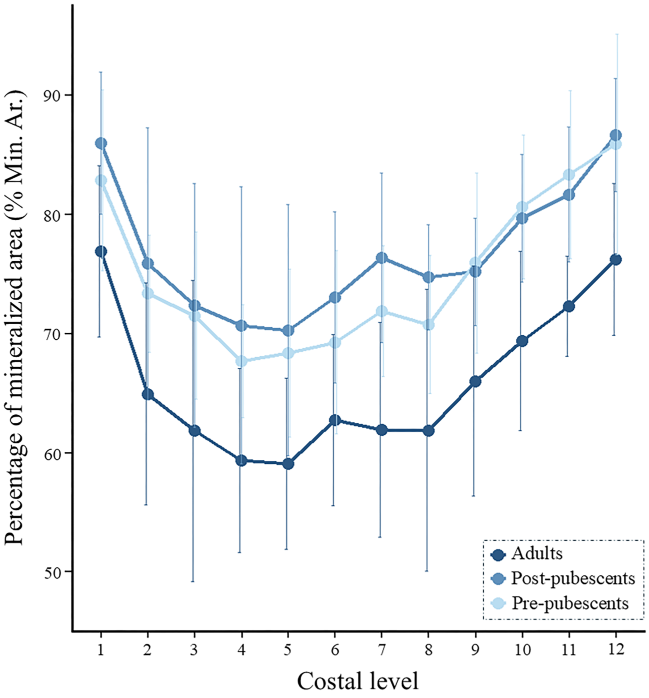

FIGURE 2 Line plots depicting the percentage of mineralized tissues of the rib cross sections. Each point represents the mean values per costal level and includes standard deviation bars.

TABLE 3 *P*-values of the permutation tests (10,000 permutations) on the percentage of mineralized area between groups per costal level.

Costal level	Pre-pubescent versus adult	Post-pubescent versus adult	Pre- versus post-pubescent
1	0.18	**0.03***	0.41
2	0.06	0.07	0.62
3	0.10	0.11	0.85
4	**0.04***	0.05	0.55
5	**0.03***	**0.04***	0.70
6	0.14	**0.02***	0.34
7	**0.03***	**0.01***	0.21
8	0.11	**0.02***	0.18
9	0.07	**0.045***	0.83
10	**0.01***	**0.02***	0.76
11	**0.01***	**0.01***	0.65
12	0.06	**0.004***	0.85

Note: Significant *p*-values are marked with an asterisk (*) and highlighted in bold.

RStudio v. 2023.12.1-402 (R Core Team, 2023) using the packages "stats" v. 4.2.3 (R Core Team, 2023) and "ggplot2" v. 3.3.3 (Wickham, 2016). The full R script is included in the Supplementary Online Material.

3 | RESULTS

Figures 2 and 3 show that adults have a lower percentage of mineralized area (%Min. Ar.) in their rib cross sections at the midshaft than both pre- and post-pubescents. This is supported by our statistical analyses since there are significant differences between the % Min. Ar. of adults and post-pubescents at costal levels 1, 5–11, and between adults and pre-pubescents at costal levels 4, 5, 7, 10, 11 (Tables 2 and 3). If we focus on differences between pre- and post-pubescents, values of % Min. Ar. are greater in post-pubescents than in pre-pubescents from costal level 1–8. Values of mineralized area for pre-pubescents are greater from costal level 9 onwards, although they are very close to post-pubescents (Figure 2). No significant differences were found between these groups at any costal level (Table 3). Regarding the tendency of the data, the three groups show a U-shaped trend with two maximum % Min. Ar. values at costal levels 1 and 12 and a minimum value at levels 4–5. However, this U-shaped trend is more pronounced in adults than in pre- and post- pubescents (Figure 1).

4 | DISCUSSION

Internal bone configuration is influenced by several factors, including physical activity and ontogeny. In relation to the latter, previous studies reported a decrease in the mineralized area of long bone cross sections during growth and development (Cambra-Moo et al., 2012, 2014; Goldman et al., 2009; Goliath et al., 2022; Pitfield et al., 2017; Swan et al., 2020). Given that this pattern has not been investigated yet in the ribs, this research aims to test for differences in the percentage of mineralized area (% Min. Ar.) in rib cross sections at the midshaft between adults, pre-pubescents, and post-pubescents. To begin with, our results show that the % Min. Ar. is significantly lower in adults compared to both pre- and post-pubescents (Figures 2 and 3, Tables 2 and 3). This is in line with previous publications on other bones (Cambra-Moo et al., 2012, 2014; Goliath et al., 2022) and occurs due to a decrease in cortical thickness followed by an increase in trabecular tissue and an expansion of its cavities.

Curiously, although there are no significant differences between pre- and post-pubescents (Table 3), the % Min. Ar. is slightly greater in the latter along most of the costal series. This result suggests that rib cross-sectional % Min. Ar. might not decrease gradually as we proposed in Hypothesis 1. Instead, it seems that there is a peak of rib cross-sectional % Min. Ar. during late puberty, which has been suggested for other parts of the skeleton (Gilsanz et al., 1988; Kröger et al., 1993; Pearson &

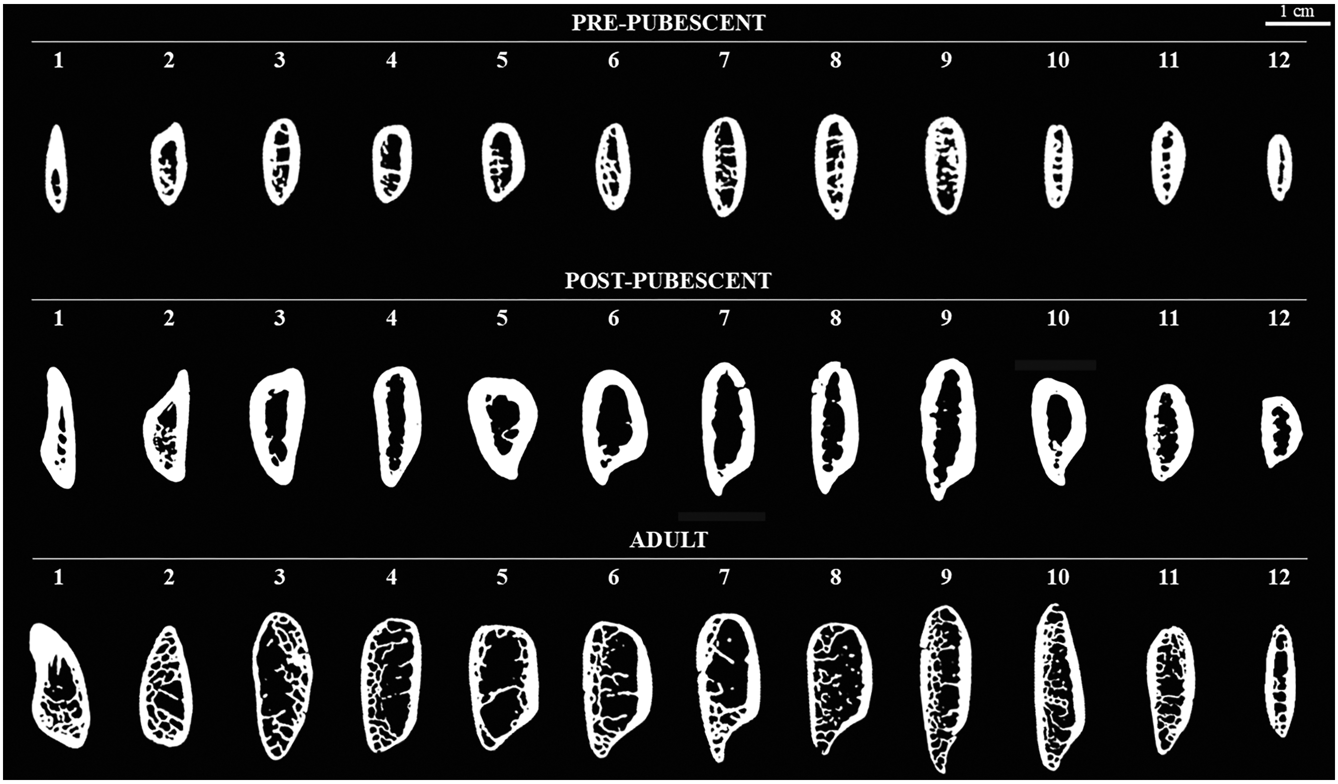

FIGURE 3 Rib cross sections extracted from the full costal series of an example individual from each group.

Lieberman, 2004). These publications state that this peak may reflect a period of accelerated bone growth that promotes the accumulation of bone mass before an eventual decline in mineralization with aging. Factors such as hormones, diet, and physical activity might modulate when this peak of bone mineralization appears, as well as its magnitude.

Regarding rib cross-sectional % Min. Ar. along the full costal series, we found that the three studied groups have the same U-shaped trend, with two maximum % Min. Ar. values at costal levels 1 and 12 and minimum value at levels 4–5 (Figure 2). This confirms Hypothesis 2, in which we proposed that the U-shaped trend found in adults (López-Rey et al., 2022) is also present in earlier stages of growth and development. We suggest that greater values are located at the beginning and the end of the costal series might respond to the mechanical stress produced by muscular attachments in these areas. Specifically, the action of the scalene muscles (ribs 1–2) and the diaphragm (ribs 7–12) during breathing might be associated to a higher % Min. Ar. in these ribs (De Troyer & Boriek, 2011; López-Rey et al., 2022, 2023). Costal levels 3–5 have the lowest % Min. Ar. values possibly because they are less actively involved in breathing kinematics (De Troyer & Boriek, 2011; Graeber & Nazim, 2007; López-Rey et al., 2022). Furthermore, the four ribs with larger % Min. Ar. in their cross sections at the midshaft are those considered "atypical" by the literature (levels 1, 2, 11, 12; Graeber & Nazim, 2007). Apart from a different shape, these ribs are notably smaller than the rest of the costal series. This observation raises the possibility that atypical ribs might somehow require higher % Min. Ar. in their cross sections to maintain structural integrity under the mechanical stress of their muscular attachments. Future studies should focus on determining whether the high mechanical stress on atypical ribs contributes significantly to the large % Min. Ar. of their cross sections or if, despite the mechanical stress, the high % Min. Ar. is primarily a result of their smaller size.

Interestingly, the U-shaped trend found for all the samples is less pronounced in pre- and post-pubescents, meaning their middle costal levels have relatively more % Min. Ar. than in adults (Figure 2). Our proposal is that this might be influenced by the fact that rib maturation occurs from the extreme to the central costal levels (Cunningham et al., 2016), so it seems that it is not until full adulthood (>30 years old) when a pronounced U-shaped trend is reached. Due to the differences observed between pre-pubescent, post-pubescent, and adult ribs, mineralization area has the potential to 1 day be used in conjunction with other osteological puberty markers, although further research is needed.

5 | CONCLUSIONS

The percentage of mineralized area (% Min. Ar.) in rib cross sections at the midshaft is lower in adults than in pre- and post-pubescents. This is consistent with previous research on long bones such as the femur and humerus. Comparing pre- and post-pubescents, the % Min. Ar. is generally greater in the latter, corresponding to the peak of bone mineralization that occurs during late puberty. Additionally, all three groups studied exhibit a U-shaped distribution of % Min. Ar. along the costal series, with higher values at the extreme costal levels, likely due to the action of the scalene muscles (ribs 1–2) and the diaphragm (ribs 7–12) during breathing. However, this tendency is less pronounced in pre- and post-pubescents, presumably because rib maturation occurs from the extreme to the central costal levels. These results are significant for disciplines such as developmental anthropology since they complement what is already known about external rib anatomical variation during growth and development. Consequently, they hold great potential for improving biological age estimation and for deepening our understanding of axial skeletal maturation in human populations.

AUTHOR CONTRIBUTIONS

J. M. López-Rey: Conceptualization; investigation; writing – original draft; methodology; visualization; writing – review and editing; software; formal analysis. **D. M. Doe:** Conceptualization; investigation; methodology; writing – review and editing; writing – original draft; supervision. **O. Cambra-Moo:** Conceptualization; writing – review and editing; visualization; validation; funding acquisition; resources; supervision; data curation; project administration. **A. González Martín:** Conceptualization; funding acquisition; writing – review and editing; visualization; validation; project administration; supervision; data curation; resources. **D. García-Martínez:** Conceptualization; investigation; funding acquisition; methodology; validation; visualization; writing – review and editing; software; formal analysis; project administration; resources; supervision.

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CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

J. M. López-Rey https://orcid.org/0000-0002-0148-5666
D. García-Martínez https://orcid.org/0000-0001-7518-3866

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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